MOLECULAR CLONING, EXPRESSION AND PURIFICATION OF A ANTIMICROBIAL TOXIN FROM THE VENOM OF THE *Lasiodora* sp. SPIDER

DUTRA A.A.A.(1), SOUSA L.O.(1), KALAPOTHAKIS E. (2), CASTRO I.M.(1)

(1) Laboratório de Biologia Celular e Molecular, Núcleo de Pesquisa em Biologia – NUPEB – Universidade Federal de Ouro Preto, (2) Departamento de Farmacologia, ICB – Universidade Federal de Minas Gerais.

Venom from snakes, scorpions, spiders and other venomous animals is a rich source of potent toxins. An interesting feature of some toxins is their remarkable species specificity. In the present work we cloned a sequence which codifies for the toxin LTx2 from the venom of the spider *Lasiodora* sp, in the expression vector pET11a. This toxin was previously identified in the screening of the cDNA library of the total venom. After cloning in expression vector we verified if the fragment was inserted in the correct frame using the Sanger DNA sequencing method. The expression of the target protein was made by adding 0,6 mM IPTG to the media followed by a 3 to 4 hours incubation. With the assistance of immunochemical assays we were detected that the protein was been expressed in the form of inclusion bodies. Then we defined a strategy to recover the soluble refolded protein. Using a solution containing 6 M urea we proceed the solubilization of the inclusion bodies. The refolding of the soluble protein was made by the dialysis method in refolding buffer. The sample was, then, submitted to HPLC, in a reversed phase column, to purify the protein. The purifying process was efficient, as shown by the Western Blotting and electrophoresis results. After that, we made an anti microbial assay, where we could see that the target protein, in the concentration of 400 mg/mL, inhibited the growth of the organisms *Escherichia coli* TOP 10F', *Salmonella agona* and *Staphilococcus epidermidis*. The expression and recovery of larger amounts of the target protein will allow tests with this toxin in other organisms and electrophysiological experiments.

KEY WORDS: Spider, *Lasiodora* sp, cloning, purification, expression, antimicrobial, toxin, venom.

FINANCIAL SUPPORT: FAPEMIG, CNPq and UFOP

CORRESPONDENCE TO: I. M. Castro, LBCM, NUPEB – ICEB-II, UFOP. Campus Morro do Cruzeiro, Ouro Preto, MG. Brazil. CEP: 35400 – 000.

TOXIC EFFECTS ON INSECTS OF VENOMS FROM Tityus metuendus AND *Brotheas amazonicus* AMAZONIAN SCORPIONS

HIGA A. M. (1,3), ARAÚJO J. DE S.(2), ARAÚJO T. F. (1,3), NORONHA M. D. N. (1,3), MARTINS MARX J.P. , MEDEIROS B. M. (4), MUNIZ, E.G. (3), AGUIAR, N. O. (5), LOPEZ-LOZANO, J.L. (1,3).

(1) Pós-Graduação em Biotecnologia – UEA; (2) Pós-Graduação em Entomologia – INPA. (3) Fundação de Medicina Tropical do Amazonas; (4) Pós-Graduação em Biotecnologia – UFAM; (5) Curso de Ciências Biológicas UFAM.

Neurotoxic peptides from scorpions venoms show depressant and excitatory effects in insects. *Tityus metuendus* is responsible of scorpionism in urban area of Manaus region and *Brotheas amazonicus* occur in Manaus region forest areas but has not medical importance. Specimens were captured in Manaus region. Venoms were collected by low voltage electric stimulation. Insect toxicity test was done in adult crickets (*Gryllus assimilis*), cockroaches (*Blaberus discoidale*) and tenebrioid beetle larvae (Zophobas morio). LD50 and PD50 were calculated by Probitos method. Venoms were inoculated in insect thoracic region and neurotoxic effects as paralysis, locomotion difficulty, body and leg trembling and death were studied. *T. metuendus* venom showed lethal activity in crickets (LD_{50} 6.3 mg) and cockroaches (LD50 74.3 mg) but with doses tested was not observed death in larvae. *T. metuendus* venom also showed fast paralyzing effects, locomotion difficulty and trembling legs in crickets (PD50 2.5 mg) but abdominal contractions with normal locomotion in cockroaches were seen, but this venom provoked fast paralysis (30 sec. approx.) in larvae. *B. amazonicus* venom showed fast paralyzing effects in crickets (PD50 2.5 mg) but in cockroach paralyzing effects were not seen. In larvae low venom doses showed fast paralyzing effect (30 sec. approx.) but with high venom doses (³ 20 µg) larvae was paralyzed by 24 hours. Our results suggest insect neurotoxins in scorpion venoms studied and different susceptibility to scorpion venoms of insect species. Molecular characterization of specifics insect neurotoxins are in course.

KEY WORDS: Amazonia, *Tityus metuendus*, *Brotheas amazonicus*, neurotoxins.

FINANCIAL SUPPORT: CAPES, FAPEAM, FMTAM, FINEP.

CORREPONDENSE TO: ANDRE MIASATO HIGA, Centro de Ofidismo Prof. Paulo Friedrich Bürnheim / FMTAM, Manaus, Brasil. Phone: + 55 92 3238 42 94. E-mail: andmhiga@yahoo.com.br.

VENOM-BASED TAXONOMY OF *Scolopendromorpha* (CENTIPEDE, ARTHROPODA)

RATES B. (1, 2), BARBOSA B. (3), MELO R. C. (4), BARBARO K. (5), KNYSAK I. (6), SILVA L. P. (7), BLOCH JR. C. (7), MORALES R. A. V. (8), BIONDI I. (9), DE LIMA M. E. (1, 2), PIMENTA A. M. C. (1, 2).

(1) Lab. de Venenos e Toxinas Animais, (2) Núcleo de Estudo de Estrutura e Função de Biomoléculas, (3) Lab. de Biodados, Biologia Celular e Desenvolvimento, (4) Lab. de Enzimologia e Fisico-Química de Proteínas, Dept. de Bioquímica e Imunologia, ICB, UFMG, Belo Horizonte, MG, Brasil. (5) Lab. de Imunopatologia, (6) Lab. de Artrópodes, Instituto Butantan, São Paulo, SP, Brasil. (7) Lab. de Espectrometria de Massa, EMBRAPA-CENARGEN, Brasília, DF, Brasil. (8) Institute for Molecular Biosciences - The University of Queensland - Brisbane, Australia. (9) Lab. de Animais Peçonhentos e Herpetologia, Departamento de Ciências Biológicas, UEFS, Feira de Santana, BA, Brasil.

Scolopendromorphs are wide-spread venomous arthropods which are responsible for a significant number of non-lethal human envenomations. Despite this, there are few specialists dedicated to the taxonomy of this group. Venom-based biochemical taxonomy has been applied to a number of animal groups. In this study, we have established a venom-based taxonomical methodology, applicable to the Scolopendromorph order. The venoms of *Scolopendra viridicornis nigra*, *S. viridicornis*, *S*. sp., *S. angulata*, *Dinocryptops miersii*, *Cryptops inheringi* and *Otostigmus pradoii* were fractionated by RPC-HPLC, and each fraction was analyzed by ESI-Q-TOF. Some fractions were also analyzed by MALDI-TOF-TOF. The data were translated into a table which contained the %ACN necessary for elution and the MW of each peptide. We have tested the feasibility of two bioinformatic approaches to extract taxonomical information from the LC/MS data: dissimilarity searching through image-analysis and machine learning-based data clustering (MLBDC) analysis. The species could be precisely grouped by the MLBDC pipeline. Therefore, this approach may complement the morphology-based taxonomy of the scolopendromorphs.

KEY WORDS: venom-based taxonomy, scolopendromorpha, LC/MS.

FINANCIAL SUPPORT: FAPEMIG, CNPq, CAPES, FAPESP.

CORRESPONDENCE TO: Adriano Pimenta. LVTA, Bloco O4, Sala 125. ICB/UFMG, Av. Antônio Carlos, 6627, 31270-901, Belo Horizonte, Minas Gerais, Brasil. Tel: + 55 031 3499-2638. apimenta@icb.ufmg.br.

Scolopendra viridicornis nigra AND *Scolopendra angulata* (CENTIPEDE, SCOLOPENDROMORPHA) VENOM PROTEOMICS

RATES B. (1,2), BEMQUERER M. P. (2,3), RICHARDSON M. (4), BORGES M. H. (1,2,4), MORALES R. A. V. (5), DE LIMA M. E. (1,2), PIMENTA A. M. C. (1,2).

(1) Lab. de Venenos e Toxinas Animais, (2) Núcleo de Estudo de Estrutura e Função de Biomoléculas, (3) Lab. de Enzimologia e Fisico-Química de Proteínas, Departamento de Bioquímica e Imunologia, Instituto de Ciências Biológicas, UFMG, Belo Horizonte, Minas Gerais, Brasil. (4) Centro de Pesquisa Professor Carlos R. Diniz, Fundação Ezequiel Dias, Belo Horizonte, MG, Brasil. (5) Institute for Molecular Biosciences - The University of Queensland - Brisbane, Australia.

Centipedes are venomous arthropods responsible for a significant number of non-lethal human envenomations. Despite this, information about the composition and function of their venom contents is scarce. In this study we made efforts to better understand the complexities of the venoms from two Brazilian centipede species (*Scolopendra viridicornis nigra* and *Scolopendra angulata*). Both venoms were analyzed through a proteomic approach which combined 2D-LC, ESI-Q-TOF/MS, N-terminal sequencing and similarity searching. Comparisons between the LC profiles and the mass compositions of the venoms of the two species are provided. Also, the N-termini of representatives of ten protein/peptide families were successfully sequenced where nine of them showed no significant similarity to other protein sequences deposited in protein databases. A screening for insecto-toxic activities in fractions from *S. viridicornis nigra* venom has also been performed. Six out of the twelve tested fractions were responsible for clear toxic effects in house flies. This work demonstrates that centipede venoms might be a neglected but important source of new bioactive compounds.

KEY WORDS: *Scolopendra*; venomic analyses; proteomics.

FINANCIAL SUPPORT: FAPEMIG, CNPq, CAPES.

CORRESPONDENCE TO: Adriano Pimenta. LVTA, Bloco O4, Sala 125. ICB/UFMG, Av. Antônio Carlos, 6627, 31270-901, Belo Horizonte, Minas Gerais, Brasil. Tel: + 55 031 3499-2638. apimenta@icb.ufmg.br.

EPIDEMIOLOGICAL AND CLINICAL ASPECTS OF SCORPION ENVENOMATION IN FORTALEZA, CEARA (BRAZIL): A RESTROSPECTIVE STUDY

ARAÚJO D.S.(2), CORDEIRO-SOUSA J.(1), MATOS E.P.N.B.(2), OLIVEIRA, R.G.S.(1), HONÓRIO JR, J.E.R.(2), FONTENELE-CARDI, N.C.(4), GUIMARÃES J.A.(3), CARVALHO K.M.(1), CARDI B.A.(1,2)

(1) Lab. Toxinologia e Farmacologia Molecular, ISCB-UECE, (2) LAPE/ISCB-UECE, (3) Centro de Atendimento a Intoxicações–CEATOX/IJF, (4) Hosp. S. José de Doenças Infecciosas, CE.

Scorpion envenomations are very common in Fortaleza, state of Ceara. In this report we presented a retrospective study based on patients data treated by CEATOX/Ceara, including epidemiological and clinical aspects of 4641 scorpion accidents from January 1999 to July 2001. Some patients brought the animal with them, and it was identified as *T. stigmurus* (TS group: 40%). The other cases, without scorpion species confirmation (60%) will be named here as OT group (OT). The majority of victims were female (60%, TS;OT). The age average was 26 to 60 (24%) and 0 to 12 (19%)for the both groups. The time spent to search medical help were about 1-2 hs. Students (33% TS; 37% OT) and house owners (26% TS; 16.5% OT) were the main victims and the main accidents occurred inside of house (93% TS; 91% OT). The parts of the body most affected were the superior members (47% TS; 47.8% OT), inferior members (44.8% TS; 44.4% OT) and back (5.8% TS; 6.0% OT). The local symptoms included: pain (65.3% TS; 64% OT), paresthesia (19% TS; 14% OT) and edema (4.4% TS; 5.0% OT). Systemic manifestations were scarce, including cephaleia (1.0% TS and OT), nausea (0.7% TS; 0.5% OT), giddiness (0.4% TS; 0.2% OT) and vomits (0.5% TS; 1.0% OT). Most of envenomation cases could be classified as mild (95%). Just a death was recorded involving a female patient that presented oedema of glottis due to unkown cause, after 9h of venom exposition, receiving 10 amp of polyvalent anti-arachnid serum. About 3.0% of cases presented no symptoms.The antiscorpion venom serum was administered in 10 patients (moderate cases). Based on these results, we could concluded that OT group probably include, mainly, *T. stigmurus* specimens, taking in consideration the similarity of the clinical manifestations. The human envenomation caused by scorpions in Fortaleza, despite frequent, could be considered benign as observed on others *T. stigmurus* endemic areas.

KEY WORDS: *Tityus stigmurus*, *Tityus* envenomation

CORRESPONDENCE TO: Bruno A Cardi (bcarditoxinol@gmail.com)

PARTIAL CHARACTERIZATION OF THE VENOM OF THE SPIDER *Oligoctenus ornatus* (CTENIDAE)

OLIVEIRA C. F. B. (1); ASSIS I. S. (1); REZENDE F. F. (1); GONÇALVES J. M. (1); SANTOS D. M. (2); BORGES M. H. (1,2); DE LIMA M. E. (2); RICHARDSON M. (1); CORDEIRO M. N. (1)

(1) Lab. de Bioquímica e Química de Proteínas, Diretoria de Pesquisa e Desenvolvimento, FUNED; (2) Lab. de Venenos e Toxinas Animais, Dep. de Bioquímica e Imunologia, UFMG

The objective of this work was to characterize the venoms of the male and female spiders of *Oligoctenus ornatus*. The venoms examined by SDS-PAGE and in reverse phase chromatography contained high complexity. The LD50 values for the female venom injected in mice (ic) were about 3.5 times more toxic than the venom of male. For flies were injected about 55ng both male and female venom and not possible to determine the LD50 value. Toxicity tests in mice showed effects such as excitability, salivation, spastic and flaccid paralysis, tail elevation and sometimes death. In flies the effects were excitability, salivation, trembling of the legs and body, lost of ability to walk or to fly and death. The crude venom of female was partially purified using reverse phase chromatography on an analytical column of Vydac C4. Preliminary bioassays with the fractions 8, 13, 17 and 40 showed toxicity towards insects. The fractions 13, 17, 22-26, 28, 29, 33, 38-41, 43 and 51 showed toxic effects in mice. SDS-PAGE zymogram experiments using hyaluronic acid, glycol chitin and gelatin as substrates showed the presence of hyaluronidase, chitinase and proteolytic enzymes, respectively in this venom. In addition, the alpha chain of fibrinogen was degraded when it was incubated with the venoms during 30 min at 37ºC. The amino acid sequences of various peptides purified from these venoms were determined by automated Edman degradation and show high similarity with peptides from *Phoneutria nigriventer* but these are other sequences that show no significant similarity.

KEY WORDS: *Oligoctenus ornatus*, spider venoms, neurotoxins, enzymes, proteolytic activity.

FINANCIAL SUPPORT: CNPq, FAPEMIG, PUC-Minas, FUNED

CORRESPONDENCE TO: CAMILA FRANCO, Lab. de Bioquímica e Química de Proteínas, Diretoria de Pesquisa e Desenvolvimento, FUNED. Belo Horizonte, MG, Brasil. Tel.: (31) 8811-1495 Email: camilafrancobo@gmail.com

ISOLATION AND PARTIAL CHARACTERIZATION OF A NEW A-NEUROTOXIN FROM *Tityus serrulatus* venom

COLOGNA C.T. (1), BERTAZZI D.T. (1), AGUIAR J.F. (2)., VARANDA W.A. (2), ARAÚJO H.S.S. (3), ARANTES, E.C. (1).

(1) Departamento de Física e Química, Faculdade de Ciências Farmacêuticas de Ribeirão Preto – USP. (2) Departamento de Fisiologia, Faculdade de Medicina de Ribeirão Preto – USP. (3) Departamento de Ciências Fisiológicas, CBME – Universidade Federal de São Carlos.

Scorpion venoms have been extensively studied in the last years because they are a good source of neurotoxins which act on ion channels. Voltage-gated Na+ channel (Nav channel) toxins are mainly responsible for the toxic effects of scorpion envenoming and can be classified into two classes: a- and b-neurotoxins. Scorpion toxins acting on Nav channels lead to an increase in Na+ influx, which in turns can induce membrane depolarization. The aim of the present study was the purification and partial characterization of a new a-neurotoxin from *Tityus serrulatus* venom (Tsv). The toxin was isolated from Tsv by ion-exchange chromatography on a 2.5 x 63.0 cm column of carboxymethyl cellulose-52, which was equilibrated and eluted with ammonium bicarbonate buffer (pH 7.8). Fraction X was submitted to a reverse-phase (C18) high performance liquid chromatography and fraction X-1 was the new a-neurotoxin. The N-terminal amino acid sequence did not show homology to previously deposited sequences. The purity of toxin X-1 was confirmed by PAGE and SDS-PAGE. In order to verify the effect of X-1 on Na+ currents, the whole cell configuration of the patch clamp technique was used on cells of the Dorsal Root Ganglion of newborn rats. X-1 (10 mM) lead to a significant increase (~90 %) in the decay time constant of inactivation without major effects on the maximal currents at each voltage. The effect is reversible upon washout of the toxin.

KEY WORDS: scorpion venom, neurotoxin, ionic channel, sodium currents.

FINANCIAL SUPPORT: CNPq.

CORRESPONDENCE TO: Camila Takeno Cologna, Faculdade de Ciências Farmacêuticas de Ribeirão Preto – USP, Av do Café, sn, Ribeirão Preto-SP. Phone: +55 16 3602 4193. Email: camilatcbio@yahoo.com.br

ANTIMICROBIAL ACTIVITY OF SEMI-ÁRIDO SPIDER HAEMOLYMPH *Acanthoscurria parahybana* (ARANEAE: THERAPHOSIDAE).

FREITAS C. I. A., PAIVA E. S., DIAS E.V., CARMO F.N. A., SERAFIM M.K.B., OLIVEIRA A.F., ROSAS N.S.C., CARMO N.A.

Laboratório de Bioagentes Patogênicos e Imunologia, Departamento de Ciências Animais, Universidade Federal Rural do Semi-Árido, Mossoró, Brasil

The reduction of success in using of antimicrobials against infection diseases causing microbes ultimately leads to an increased risk of acquiring infections in a hospital or other setting. Microbial drug resistance today is posing increasingly serious concerns. One promising answer may be new antimicrobial substances obtained of animal sources that provide the body's of defense mechanisms against pathogenic microorganisms. In this work we used haemolymph of the Brazilian semi-árido spider *Acanthoscurria parahybana* was obtained by aseptic puncture using anticoagulant rinsed material at 4°C of the dorsal tegument and immediately centrifuged (1). The supernatant were frozen until use. The susceptibility tests by disk diffusion were performed with disk diffusion method in Müeller Hinton agar against *Klebsiella pneumoniae* ATCC 10031, *Enterobacter aerogenes* ATCC 1304, *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 25922, *Salmonella choleraesuis choleraesuis* var *typhi* ATCC 6534, *Pseudomonas aeruginosa* HUWC and *Staphylococcus aureus* ATCC 6538 P. Influences of time, temperature and solubility in antimicrobial activity and toxicity using *Artemia salina* against different concentrations of haemolymph for 24 hours of evaluation also testing. In our conditions the samples of haemolimph showed a broad spectrum of antimicrobial activity, no significative toxicity and influence of stocking time and heating.

KEY WORDS: antimicrobial activity, spider haemolymph, *Acanthoscurria parahybana*

CORRESPONDENCE TO:

Carlos Iberê Alves Freitas, Laboratório de Bioagentes Patogênicos e Imunologia, Departamento de Ciências Animais, Universidade Federal Rural do Semi-Árido, BR 110 - Km 47, Bairro Pres. Costa e Silva, Mossoró - Rio Grande do Norte, Brasil, CEP 59625-900, email: ibere@ufersa.edu.br

SILVA JUNIOR, P.I. Sistema imune em aracnídeos: estrutura química e atividade biológica de peptídeos antimicrobianos da hemolinfa da aranha *Acanthoscurria gomesiana*. São Paulo, Universidade de São Paulo, Instituto de Ciências Biomédicas, 2000. 169 p. [Tese – Doutorado].

CYTOGENETIC STUDIES WITH EMBRYOS OF THE ARMED SPIDER *Phoneutria nigriventer* (KEYSERLING, 1891) (ARACHNIDA: CTENIDAE)

LAGES C.P. (1,2), SILVA L.M. (1), VALENTIM C.L.L. (3), BABA E.H. (3), FORTES-DIAS C.L.(1)

(1) Lab. Molecular and Cell Biology, Research and Development Center (DPD), Fundação Ezequiel Dias (FUNED), Belo Hte., MG; (2) Pontifícia Universidade Católica de Minas Gerais (PUC-MG), Belo Hte, MG; (3) Dep. Pharmacology, Universidade Federal de Ouro Preto (UFOP), Ouro Preto, MG, Brazil.

Phoneutria spiders are solitary and very aggressive animals, popularly known as “armed” or “banana” spiders. Amongst the four species of Phoneutria (PERTY, 1833) that occur in Brazil, *Phoneutria nigriventer* (KEYSERLING, 1891) is the most important in public health (1). Although there are several reports focusing on the biochemical and pharmacological properties of *P. nigriventer* venom, cytogenetics studies on these spiders are lacking. Aiming to identify the loci of specific toxin genes of *P. nigriventer*, we started by preparing embryonic chromosomes. Cocoons were collected at the municipality of Santa Barbara (MG) together with adult females of the same species. After external chemical cleaning, the cocoons were opened and the eggs therein were disaggregated by mechanical molecular sieving. Egg cells were immersed in CMRL medium containing colchicine and treated hypertonic 0.075 M KCl. After fixation with methanol:acetic acid (3:1), the cells suspension was dropped on glass slides and flamed for chromosome exteriorization. Staining was performed with acetic orcein and the material treated for G- and C- chromosome banding. The embryonic cells displayed 30 acrocentric chromosomes with two hyper chromic bands in the chromatids. Our next step will be devoted to the identification of the loci of specific toxin genes.

KEY WORDS: armed spider, *Phoneutria*, *Phoneutria nigriventer*, chromosome, C-banding, G-banding.

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CORRESPONDENCE TO: LUCIANA M. SILVA, Laboratório de Biologia Molecular e Celular, DCB/DPD, FUNED. Phone: 31 3371 9436; e-mail: lucianams@funed.mg.gov.br

MOVING PIECES IN A TAXONOMIC PUZZLE: VENOM 2D-LC/MS ANDA DATA CLUSTERING ANALYSIS TO INFER PHYLOGENETIC RELATIONSHIPS IN SOME SCORPIONS FROM THE BUTHIDAE FAMILY (SCORPIONES)

NASCIMENTO D.G. (1,2), RATES B. (1,2), SANTOS D.M. (1,2), VERANO-BRAGA T.(1,2), BARBOSA-SILVA A.(3), DUTRA A.A.A. (4), BIONDI I.(5), MARTIN-EAUCLAIRE M.F.(6), DE LIMA M.E.(1,2), PIMENTA A.M.C.(1,2)

(1)Lab. de Venenos e Toxinas Anim.,(2) Núcleo de Biomoléculas,(3)Lab. de Biodados, Biol. Cel. e Desenvolvimento, Depto de Bioquímica e Imunologia, ICB, UFMG, Brasil;(4)Lab. de Biol. Cel. e Molec., Depto de Farmácia, ICEB, UFOP;(5)Lab. de Anim. Peçonhentos e Herpetol., Depto de Ciências Biológicas, UEFS, Brasil;(6) CNRS-FRE 2738, Ingénierie des Protéines, UMR6560, IFR Jean Roche, Marseille, France

Taxonomical positions and phylogenetic relationships concerning representative genera and species from Buthidae family have been mostly inferred based upon morphological characters. Some authors have also compared primary structures of selected molecules found in the venoms from these scorpions. We propose a novel methodology designed to address these issues. The whole venoms from some species exemplifying peculiar cases in the Buthidae family (*Tityus stigmurus*, *T. serrulatus*, *T. bahiensis*, *Leiurus quinquestriatus quinquestriatus* and *L. q. hebraeus*), were analyzed by means of a proteomic approach using a 2D-LC/MS technique. The molecules found in these venoms were clustered according to their molecular masses and hydrophobicity index by using the Weka software. Clusters assessment was used to generate a phenetic correlation tree for positioning these species. Our results were in accordance with the classical taxonomy, which places *T. serrulatus* and *T. stigmurus* as very close species, *T. bahiensis* as a less related species and *L. q. quinquestriatus* and *L. q. hebraeus* with small differences within the same species (*L. quinquestriatus*). Therefore, we believe that this is a well-suited method to determine venom complexities that reflect the scorpions' evolutionary history, which can be crucial to reconstruct their phylogeny through the molecular evolution of their venoms.

KEY WORDS: Data clustering analyses; 2D-LC/MS; Venom proteomics; *Tityus serrulatus; T. tigmurus; T. bahiensis; Leiurus quinquestriatus*

FINANCIAL SUPPORT: FAPEMIG, MCT-FINEP, CNPq

CORRESPONDENCE TO:A.M.C.PIMENTA, apimenta@icb.ufmg.br

BEHAVIORAL REPERTORY ANALYSIS OF *Tityus stigmurus* (SCORPIONES, BUTHIDAE) IN CAPTIVITY WITH EMPHASIS IN ITS TEMPORAL PATTERNS OF ACTIVITY

ARAÚJO D.S. (2), MATOS E.P.N.B. (2), CARVALHO K.M. (1), CARDI B.A. (1,2)

(1)LABORATÓRIO DE TOXINOLOGIA E NEUROFARMACOLOGIA MOLECULAR, ISCB-UECE, (2) LABORATÓRIO DE ANIMAIS PEÇONHENTOS (LAPE), ISCB-UECE.

The study of the scorpion venoms depends, amongst other things, of significant amounts of secretion material and satisfactory techniques to the maintenance of the animals in the captivity that make possible a optimal survival of the animals. In the current study, we had as the objective to verify the variations of the behavior of the captivity of the yellow scorpion *Tityus stigmurus*, in the periods of the day and the night, objectifying to investigate the period of higher activity of the animals. The study was made in the laboratory conditions where the animals (n=6) were kept in a terrarium with typical substratum of the environment natural (land with sand and small rocks) and observed by 16 hours between 06:00 am and 00:00 am. Six categories of activities had been determined: to rest, to explore, interactions with co-specific specimens, corporal cleanness, water ingestion and hide in the burrow. During the period of the morning, immovable animals could be observed in the surface of substratum. Hidratation and the presence of the individual in its burrows had been observed generally during the period of the afternoon. During the night, it was observed higher activity of the animals with intense exploratory behavior and contact with co-specific specimens. The nocturnal behavior of *T.stigmurus* seems similar to that observed in the majority of the species of scorpions. Based on this results, we could suggest *Tityus stigmurus* must be kept in a system of inverted circadian rhythm, where we could have access the animals most active, diminishing stress caused by the handling at rest moments (during the light of the day) to increase, thus, the survival of the animals.

KEYWORDS: scorpions, behavioral repertory, *Tityus stigmurus*, temporal patterns.

FINANCIAL SUPPORT: IC/UECE

CORRESPONDENCE TO: Danilo Araújo, Laboratório de Animais Peçonhentos (LAPE), Instituto Superior de Ciências Biomédicas, UECE-Av. Paranjana, 1700, Itaperi, 60.000-000, Fortaleza, CE, Brazil. Phone: +55 85 31019824. E-mail: stigmurus@gmail.com

CAPTIVITY CONDITIONS TO IMPROVE *Tityus serrulatus* VENOM EXTRACTION

CANDIDO D.M. (1,3), KNYSAK I. (1), LUCAS S.M. (1) & BÁRBARO K.C. (2,3)

(1) Lab. Arthropods; (2) Lab. Immunopathology, Butantan Institute, São Paulo, SP; (3) Program of Post Graduation of Coord. of Control of Diseases of S. Paulo State.

Tityus serrulatus is responsible for the majority of severe cases of scorpion envenoming in Brazil. The Laboratory of Arthropods keeps a scorpion bioterium with the purpose of obtaining venom for antivenom production. The present study aims to determine which are the best captivity conditions, as well as the frequency and interval between venom extractions. Two hundred *T. serrulatus* specimens were divided in 4 groups (A, B, C and D) of 50 individuals each. A: animals not to be extracted; B, C and D extracted by electric stimulation every 30, 60 and 90 days, respectively. After 13 months of extraction, all groups had similar mortality indices (around 40%). Venom quantity averages, decreased significantly (58%) in the animals of group B, from 0.41 mg (1st month) to 0.17 mg (13th month) per animal. In Group C, the amount of venom obtained, ranged from 0.38 mg (1st month) to 0.18 mg (13th month). No decrease in venom quantity was observed in Group D. By the end of the study, group B (12 extractions) produced a total amount of 116.4 mg of dry venom, group C (7 extractions) 79.5 mg and group D (5 extractions) 75.7 mg, representing an average of 9.7 mg, 11.4 mg and 15.1 mg of poison per extraction, respectively. Different protocols do not affect scorpion survival. In case of a continuous flow of animals for this purpose, they can be extracted every month and be substituted 6 months later, when the extraction becomes unproductive. If there is no continuous flow of animals for extraction, is is recommendable to keep them in captivity for one year maximum, and extract each animal every 3 months.

KEY WORDS: captivity, *Tityus serrulatus*, venom, extraction.

CORRESPONDENCE TO: Denise M. Candido, Laboratory of. Arthropods, Butantan Institute, Avenida Vital Brasil, 1500, São Paulo, Brasil. Phone: 055XX11. 37267222 .R 2235. Email: denisecandido@butantan.gov.br

DISPERSION OF MEDICAL IMPORTANT SCORPION SPECIES IN THE STATE OF SÃO PAULO, BRAZIL

CANDIDO D.M. (1,3) & BÁRBARO K.C. (2,3)

(1) Lab. Arthropods; (2) Lab. Immunopathology, Butantan Institute, São Paulo, SP; (3) Program of Post Graduation of Coord. of Control of Diseases of S. Paulo State.

Tityus serrulatus and *Tityus bahiensis* are responsible for most of the reported accidents in Brazil. In the last few years the distribution of these animals expanded, including the urban areas of the state of São Paulo. This work aims to estimate the dispersion of *T. serrulatus* and its domain over *T. bahiensis* habitats in S. Paulo. Dispersion data was compiled from the reception of animals in the Laboratory of Arthropods (2000-2005). In the last 5 years the number of cities where scorpions were detected increased. *Tityus bahiensis* was observed in 44 cities in 2000 and increased 148% (109 cities) in 2005. *Tityus serrulatus* records increased from 18 to 91 cities (405%) in the same period. In both cases, the “Vale do Paraíba” region showed the highest number of cities with these animals. This expansion shows the great capacity of adaptation of *T. serrulatus*, a species that is not native to the area and was introduced in the state. Areas presenting sanitation problems and garbage accumulation, also favor the adaptation of these animals, which feed basically on insects. Parthenogenesis reproduction of *T. serrulatus* also collaborates to this expansion and settlement. On account of *T. bahiensis*, it is a native species of S. Paulo, but the destruction of its natural environment, associated to the sanitation problems described above, favour its dispersion.

KEY WORDS: scorpion, *Tityus serrulatus*, *Tityus bahiensis*, dispersion, São Paulo.

CORRESPONDENCE TO: Denise M. Candido, Laboratory of. Arthropods, Butantan Institute, Avenida Vital Brasil, 1500, São Paulo, Brasil. Phone: 055XX11. 37267222 .R 2235. Email: denisecandido@butantan.gov.br

HGETX1, THE FIRST K+-CHANNEL SPECIFIC TOXIN CHARACTERIZED FROM THE VENOM OF THE SCORPION *Hadrurus gertschi* *

SCHWARTZ E.F. (1, 3), SCHWARTZ C. A. (1), GÓMEZ-LAGUNAS F. (2), ZAMUDIO F. Z. (3), POSSANI L. D. (3)

(1) Department of Physiological Sciences, Institute of Biological Sciences, University of Brasilia, Brasilia, DF, 70910-900, Brazil; (2) Department of Physiology, School of Medicine, National Autonomous University of Mexico, Ciudad Universitaria, Mexico City, DF, 04510, Mexico; (3) Department of Molecular Medicine and Bioprocesses, Institute of Biotechnology, National Autonomous University of Mexico, Cuernavaca, Morelos, 62210, Mexico

A novel K+-channel toxin was identified, purified and characterized from the venom of the scorpion *Hadrurus gertschi* (abbreviated HgeTx1). It is a 36 amino acids long peptide, has a molecular mass of 3950 atomic mass units (a.m.u.) and contains four disulfide bridges established between Cys1-Cys5, Cys2-Cys6, Cys3-Cys7 and Cys4-Cys8. HgeTx1 blocks reversibly the Shaker B K+-channels with a Kd of 52 nM. It shares 60, 45 and 40% sequence identity respectively with *Heterometrus spinnifer* toxin1 (HsTX1), *Scorpio maurus* K+-toxin (maurotoxin) and Pandinus imperator toxin1 (Pi1), all four-disulfide bridged toxins. HgeTx1 is 57-58% identical with the other scorpion K+-channel toxins that contain only three disulfide bridges. Based on sequence comparison, chain length and number of disulfide bridges analysis we classify HgeTx1 into subfamily 6 of the α-KTx scorpion toxins (systematic name: α-KTx 6.14).
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KEY WORDS: Amino acid sequence, *Hadrurus gerts*chi, HgeTx1, K+ channel, scorpion toxin, Shaker B

FINANCIAL SUPPORT: CNPq, DPP-FUB, CONACyT, DGAPA-UNAM

CORRESPONDENCE TO: Elisabeth F. Schwartz, Departamento de Ciências Fisiológicas, Instituto de Ciências Biológicas, Universidade de Brasília, Brasília, DF, 70910-900, Brasil, Phone: 55-61-33072160, Fax: 55-61-32741251, E.mail: efschwa@unb.br

VENOM PRODUCTION OF SPIDERS GENUS Loxosceles HEINECKEN & LOWE, 1839 (ARANEAE; SICARIIDAE): VARIATIONS RELATED TO THE SPECIES, SEX AND SEQUENTIAL EXTRACTIONS

MARQUES-DA-SILVA E.(1) & FISCHER M. L.(2)

(1) Seção de Animais Peçonhentos, Centro de Produção e Pesquisa de Imunobiológicos – SESA - Paraná; (2) Núcleo de Estudos do Comportamento Animal – CCBS – PUCPR.

Venomous spiders genus *Loxosceles* cause thousands of accidents all of the years in the Paraná state, Brazil. The high incidence of Loxoscelism in this state led to the production of loxoscelic antivenom by the Center of Production and Research of Imunobiologicals (CCPI). The Loxosceles spiders` venom was obtained by electric stimulation and crystallization by vacuum desiccation. Our objective was to analyze the variation in the productivity of the venom of females, males and juveniles (300 *L. intermedia*, 194 *L. gaucho* and 273 *L. laeta*) in successive extractions with a time interval of 20 days. Among the species more venom was extracted from *L. laeta* (0,075 mg/spider), followed by *L. gaucho* (0,066 mg/spider) and *L. intermedia* (0,058 mg/spider). For *L. intermedia* the female (0,09 mg/spider) produces 3 times more venom than males and juveniles; for L. gaucho the female (0,102 mg/spider) produce 2,3 times more venom than male and 5,6 times more than juveniles; for *L. laeta* the female (0,113mg/spider) produce 4 times more venom than males e 2,6 times than juveniles. The greater resistance to sequential extractions was shown by *L. laeta* being possible to extract up to 5 times (respectively 55%, 52%, 46% and 28% of survival rate). Individuals *L. intermedia* had their venom extracted up to 3 times (respectively 54% and 74% of survival rate); *L. gaucho* was extracted only 2 times (32% of survival rate). Any of the three species showed decrease in the average venom production per spider in successive extractions. Venom production of the *Loxosceles* is related to the relative amount of males used, being related to the low amount of venom liberated by the male and with their low resistance to the electric stimulation.

KEY WORDS: spider, venom, *L. gaucho*, *L. intermedia*, *L. laeta*.

CORRESPONDENCE TO: EMANUEL MARQUES DA SILVA, Seção de Animais Peçonhentos, Centro de Produção e Pesquisa de Imunobiológicos (CPPI). R: Targino da Silva s/nº, Piraquara – Paraná – Brasil. CEP: 83302-160. Email: ems@pr.gov.br

ACCIDENTAL DISCOVERY OF A TACHYLECTIN-LIKE PROTEIN, WHICH HAS HOMOLOGY TO FIBRINOGEN, IN THE VENOM FROM THE BRAZILIAN SPIDER *Phoneutria nigriventer*

REZENDE, F. F.(IC); ASSIS, I.S.(IC); OLIVEIRA, C. F. B.(IC); GONÇALVES, J. M.(AT); BORGES, M. H.(PD); RICHARDSON, M.(PD); CORDEIRO, M. N.(PD).

Laboratório de Bioquímica e Química de Proteínas, Diretoria de Pesquisa e Desenvolvimento, Fundação Ezequiel Dias – Belo Horizonte, MG.

In this work whilst attempting to purify the enzyme hyaluronidase from the venom of the spider *Phoneutria nigriventer* using gel filtration (Sephadex G-50) and reverse phase HPLC (Vydac C4) we accidentally discovered a dimeric lectin of 52kDa. This is the first report of any such molecule in the venom of the order Araneae. This lectin has an integrin-binding domain (RDG) and an amino acid sequence which shows 54% and 45% identity with the Tachylectins (TL) 5A and 5B respectively, which are isoforms isolated from the hemolymph of the horseshoe crab *Thachypleus tridentatus*. The TLs are lectins which recognize specific polysaccharides of pathogens and are involved in the innate immunity of the organism. In addition, the Tachylectins have homology with mammalian ficolins and vertebrate fibrinogen, with the functions of recognition and agglutination, respectively.

Considering the structure and possible functions of the lectin from spider venom, it appears to have been derived from a multi-functional molecule, which perhaps was a common ancestor of immune and aggregation systems, found in invertebrates and vertebrates.

KEY WORDS: *Phoneutria nigriventer*, lectins, fibrinogen, venoms, Tachylectin.

FINANCIAL SUPPORT: FAPEMIG, CNPq, PUC-Minas, FUNED.

CORRESPONDENCE TO: FLÁVIA FIGUEIREDO DE REZENDE, Laboratório de Bioquímica e Química de Proteínas, Diretoria de Pesquisa e Desenvolvimento, Fundação Ezequiel Dias. Belo Horizonte, MG, Brasil. Tel.: (31) 33719431/92813403. Email: rezendeff@gmail.com

PURIFICATION, MOLECULAR MASS AND N-TERMINAL DETERMINATION OF AN INSECTICIDAL TOXIN FROM THE VENOM OF THE *Grammostola iheringi* (MYGALOMORPHAE: THERAPHOSINAE)

MONTANDON G. G. (1,2,3), SANTOS D. M. (2,3), FERRAZ, K. K. F. (2,3), BEMQUERER, M. P. (3), PIMENTA, A. M. C. (2,3), MONTENEGRO, F. C. (4), LUCAS, S. M. (5), CORDEIRO, M. N. (6), De LIMA M.E. (2,3), DINIZ M.R.V. (7).

(1) Bolsista Programa de Educação Tutorial (PET– MEC/SESu) Ciências Biológicas da Pontifícia Universidade Católica de Minas Gerais (PUC Minas); (2) Laboratório de Venenos e Toxinas Animais (LVTA); (3) Núcleo de Biomoléculas; (4) Laboratório de Membranas Excitáveis, Depto. Bioquímica e Imunologia, ICB, UFMG; (5) Laboratório de Artrópodes, Instituto Butantan; (6) Laboratório de Bioquímica e Química de Proteínas, Fundação Ezequiel Dias (FUNED); (7) Laboratório de Biologia Molecular II, Fundação Ezequiel Dias (FUNED).

Venomous animals have evolved a vast array of peptide toxins for prey capture and defense. These peptides are directed against a wide variety of pharmacological targets, making them an invaluable source of ligands for studying the properties of these targets in different experimental paradigms. In this respect, spiders as a highly diversified group of almost exclusive insect predators appear to possess great potential for the discovery of novel insect-selective toxins. Only the venom of a tiny percentage of the 40.000 known spiders have been well characterized. In this work, we fractionated the crude venom of Grammostola iheringi by RP-HPLC which yielded 40 fractions that were collected manually and assayed for paralytic activity for house fly (Musca domestica). Seventeen fractions were toxic to insects, being two of them further purified and characterized. The biochemical characterization involved the N-terminal sequence - SXQQKWMWTXGQQX - established by direct automated Edman degradation, and the molecular mass of 3587 Da determined by mass spectrometry. BLAST searches revealed similarity with spider potassium channel inhibitory toxin family and this result was confirmed by tests in channels Kv1.3 from L929 cells.

KEY WORDS: spider, spider venom, *Grammostola iheringi*, insecticidal toxin.

FINANCIAL SUPPORT: Fapemig, CNPq

CORRESPONDENCE TO: Dr. Marcelo Ribeiro Vasconcelos Diniz. E-mail: mdiniz@funed.mg.gov.br

CONSTRUCTION OF A NEW DATA MODEL FOR STORING AND RETRIEVING TOXIN INFORMATION

FARIA-CAMPOS A. C. (1), RATES B.(2,3), GOMES R. R. (1), FERNANDES-RAUSCH H. (4), MORATELLI F. S. (1), DE LIMA M. E. (2,3), PIMENTA A. M. C. (2,3), CAMPOS S. V. A. (4), FRANCO, G. R. (1).

(1) Lab. de Genética Bioquímica, (2) Lab. de Venenos e Toxinas Animais, (3) Núcleo de Estudo e Função de Biomoléculas, Dep. de Bioquímica e Imunologia, ICB, (4) Lab. de Universalização de Acesso a Internet, Dep. de Ciência da Computação, ICEx,UFMG.

Experiments regarding the analysis of toxins from the poisonous arthropod *Scolopendra viridicornis* (Centipede, Scolopendromorpha) have been performed by members of our group resulting in the production of an expressive amount of data. The analysis of this data has been made manually and the data stored in flat-files or formats associated to a given HPLC or MS equipment. This implies in an increased time for data retrieval and limits data integration to the relationships assigned manually by the researcher. Furthermore, there is no automatic integration between experimentation, analysis and previously existing data. In this work we propose the construction of a data model to store proteomic data using a relational database. The use of a relational database will allow us to store raw and processed data and to represent their relationship. By using this we intend to enable a semi-automatic analysis, recording results in the database and notifying researchers when certain criteria have been met, such as the identification of compounds with the same molecular mass in different experiments. The database construction using a relational approach speeds up the whole process of toxin identification, since it makes explicit associations provided by the researcher at different moments or from experiments of different types. The proposed model uses groups of tables for each data subtype, which store raw data, details regarding the experimental procedure, analysis results and linked publications. The model has been implemented using MySQL under a Linux platform and harbors data from S. viridicornis and other poisonous arthropods. Using this data model we hope to contribute for the study of animal toxins by making the analysis of toxin data easier, faster and more precise.

KEY WORDS: *Scolopendra*, toxin, database, proteomics.

FINANCIAL SUPPORT: CAPES, CNPq, FAPEMIG.

CORRESPONDENCE TO: Alessandra Faria-Campos. alessa@icb.ufmg.br

IDENTIFICATION AND PURIFICATION OF ENZYMES FROM THE VENOM OF THE SPIDER *Phoneutria keyserlingi (*CTENIDAE)

ASSIS SOUZA, I.; REZENDE, F. F.; OLIVEIRA, C. F. B.; GONÇALVES, J. M.; BORGES, M. H.; RICHARDSON, M.; CORDEIRO, M. N.

Laboratório de Bioquímica e Química de Proteínas, Diretoria de Pesquisa e Desenvolvimento, Fundação Ezequiel Dias

Spiders of the genus *Phoneutria* are frequently found near human habitations and are often involved in envenenomations. In this work, we investigated the enzymatic profile in the venom of the spider *Phoneutria keyserlingi*. SDS-PAGE zymogram experiments using hyaluronic acid and gelatin as substrates showed the presence of hyaluronidase and proteolytic enzymes in this venom. In addition, the alpha chain of fibrinogen was degraded when it was incubated with the venom during two hours at 37^{o}C, confirming the proteolytic activity. The crude venom was partially purified by reverse phase chromatography on a preparative column of Vydac C4. The hyaluronidase and proteolytic activities were determined in each of the fractions collected towards the end of the gradient of acetonitrile. The fraction 38 which was eluted with 47% acetonitrile-TFA 0.1%, showed both proteolytic and fibrinogenolytic activity and the fractions 39-40 (49%), also showed hyaluronidase activity. The enzyme hyaluronidase found in the venom of many spiders acts as a spreading factor for the toxins because it degrades hyaluronic acid, a glycosaminoglycan in the extracellular matrix of connective tissues. So far there are no details available of the structure of this enzyme from the venom of any member of the sub-phylum Arachnida. We are currently completing the purification of hyaluronidase and the proteinases with the objective of determining their primary structure.

KEY WORDS: *Phoneutria keyserlingi*, enzymes, hyaluronidase, venoms, proteolytic activity.

FINANCIAL SUPPORT: FAPEMIG, CNPq, PUC-Minas, FUNED

CORRESPONDENCE TO: IVANA ASSIS, Laboratório de Bioquímica e Química de Proteínas, Diretoria de Pesquisa e Desenvolvimento, Fundação Ezequiel Dias. Belo Horizonte, MG, Brasil. Tel.: (31) 8865-0923 Email: souzaivana@gmail.com

PRELIMINARY TOXINOLOGICAL CHARACTERIZATION OF THE VENOM OF THE SCORPION *Centruroides margaritatus* (BUTHIDAE, GERVAIS, 1841) OF THE VALLE OF THE PATÍA, COLOMBIA

GUERRERO-VARGAS J.A. (1,2), AYERBE S. (2,5), RADA- MENDOZA M. (4), VÉLEZ P. (5), BELTRAN J. (5), D´SUZE G. (6), FONTES W. (1), SOUSA M.V (1) AND CASTRO M.S. (1,3)

(1) Brazilian Center for Protein Research, University of Brasilia, Brasilia/DF, Brazil; (2) Group of Herpetological and Toxinological Research, Museum of Natural History University of Cauca, Colombia; (3) Laboratory of Toxinology, University of Brasilia Brasilia/DF, Brazil; (4) Chemistry Department, University of Cauca, Colombia; (5) Department of Biology, University of Cauca, Colombia; (6) Venezuelan Institute of Scientific Researches, Venezuela.

The venom of the scorpion *Centruroides margaritatus* of the Valle of the Patía Colombia is toxic to human and is able to engender serious complications. Venom components and its effects are not very well-known. In this study the scorpion venom was separated, in first instance, by HPLC using a Protein Pak exclusion column. Nine fractions were obtained and the fractions II, III, IV and V were tested in gastrocnemius muscle of *Bufo marinus*. The fractions II, IV and V inhibited the muscular contractions, whereas the fraction III did not cause any effect. Excitability, salivation, dyspnea and sialorrhea were observed after administration of the crude venom in mice. The venom production in milligrams of protein for this species is low and corresponds to 0.11 mg by individual. The LD50 determined by the method of Sevcik was 42.83 mg/kg. A proteomic approach was also applied to this scorpion venom: the crude venom was submitted to a reversed-phase chromatography (Vydac C8 4.6 x 250 mm) and 77 eluted fractions were collected and analyzed by MALDI-TOF mass spectrometry (Reflex IV, Bruker). One eluted fraction with a molecular mass of 2,6 kDa was submitted to Edman degradation and its partial sequence exhibits similarities to other scorpion potassium blocking neurotoxins.

KEY WORDS: scorpion, *Centruroides margaritatus*, Patía, Colombia, biological assays, proteomic analysis.

FINANCIAL SUPPORT: FUB/UnB

CORRESPONDENCE TO: Mariana S. Castro (E-mail: mscastro@unb.br) or Jimmy Alexander Guerrero Vargas (E-mail: guerrero@unb.br), Brazilian Center for Protein Research, University of Brasilia, Brasilia/DF, Brazil.

THE EFFECT OF TX2-6 TOXIN OF *Phoneutria nigriventer* SPIDER IN ERECTILE FUNCTION IN RATS

NUNES K.P. (1), COSTA-GONÇALVES A. C. (1), LANZA L. F. (1), CORTES S. F. (1), CORDEIRO M. N. (4), RICHARDSON M. (4), PIMENTA A. M.C. (3), LEITE R. (2), DE LIMA M.E. (3)

(1) Department of Physiology and Pharmacology, UFMG; (2) Department of Physiology, Medical College of Georgia, U.S.A; (3) Department of Biochemistry and Immunology, UFMG, Brazil. (4) FUNED, Foundation Ezequiel Dias, MG, Brazil.

Severe human accidents involving the venom of spider *Phoneutria nigriventer* are characterized by different symptoms including priapism. The aim of this study is to investigate the role of the toxin Tx2-6, a peptide extracted from the venom of *P. nigriventer*, in the penile erection caused by the spider poisoning. Erectile function was measured in urethane anesthetized Wistar rats (230-250g) by continuous monitoring mean arterial pressure (MAP) and intracavernosal pressure (ICP) during electrical stimulation of the major pelvic ganglion. Voltage-response curves (0.5-3.0V, 12Hz, 0.1ms, 30s each step) were performed before and (15 min) after subcutaneous injection of Tx2-6 (12 µg/Kg). The erectile response induced by ganglionic stimulation was significantly potentiated after injection of Tx2-6. Treatment with L-NAME (200 mg/Kg, i.c.), a non selective NOS (nitric oxide synthase) inhibitor, significantly impaired the erection, and this effect was not overcome by the treatment with Tx2-6. Incubation with Tx2-6 (0,1 and 0,01µg/mL) caused a significant release of NO from rat isolated strips of *corpus cavernosum*, detected through a NO indicator (DAF-FM) using confocal microscopy. This study indicates that Tx2-6 is probably the component of the venom responsible for the priapism observed in poisoning accidents with the *P. nigriventer* spider, and that Tx2-6 induces and or potentiates penile erection by facilitating the nitric oxide -cyclic GMP pathway.

KEY WORDS: *Phoneutria nigriventer*, Tx2-6 toxin, nitric oxide, erectile dysfunction

FINANCIAL SUPPORT: CNPq

CORRESPONDENCE TO: DE LIMA MARIA ELENA, Laboratory of Poison and Animals Toxins, LVTA, Belo Horizonte, Brazil. Phone: +55+31+3499 26 59. Fax: +55+31+3499 26 74. Email: delima@icb.ufmg.br

COMBINING A NEW BIOINFORMATIC TOOL AND EXPERIMENTAL APPROACHES TO MAP A NEUTRALIZING EPITOPE ON THE *Loxosceles intermedia* PROTEIN 1 (LiD1)

FELICORI L.F (1,2), MOURA J.F.(1), MOREAU V. (2), KALAPOTHAKIS E. (3), ALVARENGA L.M. (1,2), CHAVEZ-OLORTEGUI C. (1), MOLINA F. (2), GRANIER C. (2)

1- Departamento de Bioquimica e Imunologia, UFMG, Belo Horizonte, Brazil

2- Centre de Pharmacologie et Biotechnologie pour la Santé, Montpellier, France

3- Departamento de Biologia Geral, UFMG, Belo Horizonte, Brazil

Bites of the spider *Loxosceles* sp lead to dermonecrotic and systemic effects in humans. In the venom, the group of dermonecrotic factors (DNF) is responsible for the toxic activity. The monoclonal Limab7 was able to neutralize dermonecrotic activity. To better understand the molecular mechanism of neutralization of this antibody, we combined bioinformatics and biological experiments to identify the epitopic region of the *Loxosceles intermedia* dermonecrotic factor (LiD1) bound by Limab7. To that end, sets of immobilized 15- and 25-mer overlapping peptides covering the complete amino acid sequence of LiD1 were synthesized and their reactivity with Limab7 mesured. No reactivity was observed. By using a peptide phage-display technology, we selected four different peptides (mimotopes) able to bind to Limab7. They, however, showed no apparent similarity with the LiD1 sequence, indicating that epitope residues are non contiguous. The mimotopes sequences were then analysed by MIMOP, a novel bioinformatic tool using the three-dimensional model of the protein. This analysis disclosed nine amino acids at the surface of LiD1 as likely candidates for the epitopic region. Two residues belong to the active site of LiD1, which is consistant with the neutralizing properties of Limab7. In order to validate this epitope prediction, we are now performing site-directed mutagenesis of six different amino acids of the LiD1 protein.

KEY WORDS: spider venom, neutralizing monoclonal antibody, epitope mapping, mimotope, mutagenesis, bioinformatics

FINANCIAL SUPPORT: CAPES- COFECUB, CNPq, FAPEMIG

CORRESPONDENCE TO: Liza Felicori, Departamento de Bioquimica e Imunologia, UFMG, Belo Horizonte, MG, Brazil. E-mail: liza@icb.ufmg.br

STUDY OF THE VENOM OF *Tityus serrulatus* (SCORPIONES; BUTHIDAE) FROM TWO REGIONS OF THE STATE OF BAHIA, BRAZIL

SILVA T.F. [1]; LIRA-DA-SILVA R. M.[1]; CASAIS-E-SILVA L. L.[2]

(1) Núcleo de Ofiologia e Animais Peçonhentos da Bahia (NOAP); Instituto de Biologia; Universidade Federal da Bahia; (2) Núcleo de Estudos de Venenos Animais; Fundação Bahiana para Desenvolvimento das Ciências (NEVA/FBDC)

The *Tityus serrulatus* scorpion causes most accidents and induces the most serious forms of poisoning in Brazil. The present was designed to characterize the *T. serrulatus* venom (0.4 or 0.8 mg/Kg) (TsV) from the Region Metropolitan of Salvador (RMS) and the Southwestern Region of the State of the Bahia (RSB), Brazil to investigate whether there is regional variation of this specie. Male Swiss mice (18-22g) was used to evaluate the toxicity for the FINNEY method (1971). Pulmonary edema was calculated by the difference of humid weight of the lung of control and experimental rats. The neurotoxic activity was determined by observation of behavioural of the animals after envenomation. The statistics analysis was performed by ANOVA ($P<0.05$) using the program Graphpad InStat®. The calculated LD50 was 96.3 µg/mice for RMS and 39.1 µg/mice for the RSB. These results showed low toxicity compared to TsV from other regions of Brazil. Our results also demonstrated that the venom of both regions do not induce pulmonary edema, as assessed by the evaluation of the index lung/corporal weight as the histological evaluation. Mice injected intravenously presented similar autonomic signs for both venoms. Only the presence of aggressiveness and absence of sialorrhea and ocular congestion were observed for venom of Southwestern Region. It is concluded that TsV from Bahia State shows low toxicity and do not induces lung edema. Our data suggest that these features seem contribute to the absence of death for the poisoning for *Tityus serrulatus* registered in the Region Metropolitan of Salvador.

KEY WORDS: scorpion, venom, *T.serrulatus*

CORRESPONDENCE TO: Tiago Ferreira da Silva; tiagofs@ufba.br; Luciana L. Casais e Silva; casais@uneb.br; Rejane M. Lira da Silva; rejane@ufba.br

DEVELOPMENT OF THE VENOM APPARATUS IN THE ARMED SPIDER, *Phoneutria nigriventer* (KEYSERLING, 1891): STRUCTURAL AND MICROANATOMICAL ANALYSIS

SILVA L. M. (1, 2, 3), BOTELHO A. C. C. (3), PIMENTA P. F. P. (3), FORTES-DIAS C. L. (1)

(1) Lab. Molecular and Cell Biology, Research and Development Center (DPD), FUNED, Belo Hte, MG, Brazil; (2) Dep. Morphology, UFMG, Belo Hte, MG; (3) Lab. Medical Entomology, CPqRR/FIOCRUZ, Belo Hte, MG, Brazil.

The venom of *Phoneutria nigriventer* spiders is composed of a variety of bioactive substances. It has been considered as a rich source of potential candidates for the development of new drugs for prevention/treatment of a number of physio-pathological conditions or for agricultural use as natural insecticides. Although the venom composition has been extensively studied, secretion pathways are poorly understood. In this study, the presence of venom glands at early development stages of *P. nigriventer* was investigated. The ultra structure and microanatomy of four intra-cocoon stages (egg, first pre-larva, second pre-larva and pre-nymph) were analyzed by scanning electron (MEV) and confocal laser microscopy (CLM). The samples were fixed with 2.5% glutaraldeyde, 4% sucrose in 0.1 M cacodylate buffer (MEV) or with 4% paraformaldeyde (CLM). Our results demonstrated that the venom glands are among the first organs to be formed in *P. nigriventer* spiders. They are protected by a double layer of muscle fibers that contracts for venom release. Possibly, this external layer may avoid, also, the toxic action of the stored secretion on the physiological system of the spider itself, considering the known action of the venom toxins on invertebrates. At the pre-nimph stage, the venom apparatus is fully developed showing a pair of chelicera and stingers. Molecular studies are in progress to check for the presence of specific toxins in the venom glands of *P. nigriventer* spiders at early development stages.

KEY WORDS: *Phoneutria nigriventer*, armed spider, venom gland, MEV, CLM.

FINANCIAL SUPPORT: CNPq and FAPEMIG

CORRESPONDENCE TO: LUCIANA M. SILVA, Lab. Biologia Celular/DPD, FUNED. R. Conde Pereira Carneiro 80, 30510-010, Belo Hte, MG. E-mail: lucianams@funed.mg.gov.br

PURIFICATION AND CYTOTOXIC CHARACTERIZATION IN MYOBLASTS AND MYOTUBES (C2C12) OF THE NEW FACTOR FROM *Tityus serrulatus* SCORPION VENOM

PONCE-SOTO L.A. (1), NOVELLO J.C. (1), MARANGONI S. (1)

(1) Department of Biochemistry, Institute of Biology, State University of Campinas, P.O. Box 6109, Zip code 13083 970, Campinas, SP, Brazil.

The new factor protein, was purified from *Tityus serrulatus* scorpion venom after one chromatographic step, reverse phase HPLC on μ-Bondapack C-18. The molecular mass by SDS-PAGE was 4325.34 Da and confirmed by MALDI-TOF mass spectrometry .The amino acid composition showed that new factor have a high content of Lys, Asx, Gly, and 8 half-Cys residues, typical of a basic toxins from scorpions. Cytotoxic activity of new factor from *Tityus serrulatus* scorpion venom was assayed on murine skeletal muscle C2C12 myoblasts and myotubes. Variable amounts of toxin were diluted in assay medium (Dulbecco´s Modified Eagle´s Medium supplemented with 1% fetal calf serum) and added to cells in 96 well plates, in 150 ml. Controls for 0 and 100% toxicity consisted of assay medium, and 0.1% Triton X-100, respectively. After 3 h at 37 ºC, a supernatant aliquot was collected for determination of lactic dehydrogenase (LDH; EC 1.1.1.27) activity released from damaged cells, using a colorimetric end-point assay(Sigma 500C). Experiments were carried out in triplicate. The whole venom *in vitro*, did not lyse skeletal muscle myoblasts and myotubes, with a dose of 1.0 µg/well (1.0 µg/150 µl), in contrast the new factor lysed skeletal muscle myoblasts and myotubes, with a dose of 1.0 µg/well (1.0 µg/150 µl) causing 100% cytotoxic suggesting that the cytotoxic action of venom is strongly influenced by the new factor. The new factor displays a more restricted cytotoxic profile on cells in culture, mainly affecting differentiated skeletal muscle myotubes, which are cytotoxic on differentiated myotubes most but that in on myoblasts. Interestingly, the new factor, exerts a wider cytotoxic effect, suggesting an interaction with widely distributed targets in the membranes of other cells.

KEY WORDS: *Tityus serrulatus*, HPLC, Myoblasts, Myotubes and Cytotoxicity.

FINANCIAL SUPPORT: CAPES, FAPESP, CNPq

CORRESPONDENCE TO: Ponce-Soto, Luis Alberto Phone: + 19 35216132. Email: poncesoto@yahoo.com.ar

COMPARATIVE STUDIES AMONG BRAZILIAN CENTIPEDE VENOMS

LIRA M. S. (1), MALTA M. B. (1), SOARES S. L.(1), ROCHA G. C. (1), KNYSAK I. (2), MARTINS R.(2), GUIZZE S. P. G.(2), SANTORO M. L. (3), BARBARO K. C. (1)

(1) Lab. of Immunopathology; (2) Lab. of Arthropods, (3) Lab. of Pathophysiology, Butantan Institute, SP, Brazil.

Envenomation by centipedes is characterized by burning pain, paresthesia and edema, sometimes evolving to superficial necrosis. These animals have a pair of forceps connected to a venom gland used to arrest the prey. They prefer lo live in hidden places, which allow them an easy adaptation around and inside residences in urban areas. The aim of this work was to compare some properties of *Otostigmus pradoi*, *Cryptops iheringi* and *Scolopendra viridicornis* centipede venoms. By SDS-PAGE (4-20%), differences were noticed among venoms (between 7 and 205 kDa). A strong band (around 130 kDa) was similarly detected in all venoms. Using zymography, caseinolytic (around 40 kDa) and gelatinolytic activities (distributed between 16 and 190 kDa) were observed in all venoms. Components with fibrinogenolytic (approximately 40 kDa) and hyaluronidase (42 kDa region) activities were only observed in S. viridicornis and O. pradoi venoms. Most of these enzymatic components are metalloproteinases. Cross-reactivity was detected among all venoms by ELISA and Western blotting using species-specific sera raised in rabbits. All venoms induced nociception, edema and myotoxicity in mice, but only *S. viridicornis* induced discrete hemorrhagic activity. No coagulant activity was detected in centipede venoms and only *S. viridicornis* had direct hemolytic activity on human O positive red blood cells. Discrete differences were noticed among centipede venoms, but *S. viridicornis* showed the most toxic activities, which can explain the severity of the clinical picture observed in human accidents by this species.

KEY WORDS: centipede, venoms, *Scolopendra viridicornis*, *Cryptops iheringi*, *Otostigmus pradoi*.

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CORRESPONDENCE TO: KATIA CRISTINA BARBARO, Av. Vital Brazil 1500, 05503-900, São Paulo, SP, Brazil, Telephone: +55 11 3726 7222 ext. 2278/2134. Email: kbarbaro@butantan.gov.br.

HIPPOCAMPAL EFFECTS OF A TOXIN ISOLATED FROM *Tityus serrulatus* SCORPION VENOM: A BEHAVIOURAL, ELETROENCEPHALOGRAFIC AND HISTOPHATOLOGIC STUDY

FREITAS M. M (1)., DORCE V.A.C.(1), LEBRUN I.(2)., NENCIONI A.L.A. (1)

(1) Pharmacology, (2) Biochemistry and Biophysic Laboratory, Butantan Institute, São Paulo – Brasil.

Scorpion venoms are composed among other substances by neurotoxins that act on ionic channels, mainly sodium and potassium. Previous studies showed that some toxins of *Tityus serrulatus* venom (vTs) have epileptic and neurotoxic effects. The aim of this study was to investigate the effects of IV–IV toxin isolated from this venom in the hippocampus of rats. Male Wistar rats (220 – 250g) were anesthetized and positioned in a stereotaxic frame. Stainless steel guide cannulas and bipolar twisted electrodes were chronically implanted in the hippocampus. One day after surgery the animals were injected with 1µg/µl of toxin (n=6) or Ringer solution (control group, n=6). After the injections, continuous eletroencephalografic recording (EEG) and observations of animals behavior were performed for periods of 4h. Seven days after the injections the animals were sacrificed and perfused. The brains were removed and prepared for histological analysis. The EEG showed intense epileptic-like discharge often accompanied by behavioral alterations as wet dog shake and mioclonia. The histopathological analysis showed neuronal death in CA1, CA3 and CA4 ipsilateral to injection and in CA4 contralateral. The toxin IV–IV causes neuronal death and has convulsive effect.

KEY WORDS: *Tityus serrulatus*, scorpion toxins, hippocampus, Neurodegeneration.

FINNANCIAL SUPPORT: Fundação Butantan, CCD -SES - SP

CORRESPONDECE TO: Freitas, Marcela de M., Butantan Institute, São Paulo – Brasil. Phone: + 11 37267222 ramal: 2299. Fax: 37267222 ramal: 2133. E-mail: marcelamfreitas@hotmail.com

PURIFICATION OF HYALURONIDASE AND FIBRINOGENOLITIC ENZYMES FROM THE VENOM OF THE BRAZILIAN SCORPION *Tityus serrulatus*

BORGES M. H. (1,2), ASSIS I. S. (1), REZENDE F. F. (1), OLIVEIRA C. F. B. (1), GONÇALVES J. M. (1), BATISTA C. R(1), PIMENTA A.M.C. (2), NASCIMENTO D.G. (2), RICHARDSON M. (1), CORDEIRO M. N. (1), DE LIMA M.E. (2)

(1) Lab. de Bioquímica e Química de Proteínas, Diretoria de Pesquisa e Desenvolvimento, FUNED, (2) Lab. De Venenos e Toxinas Animais-LVTA, Departamento de Bioquímica - UFMG

Arthropod venoms are very complex mixtures of molecules, including peptides, proteins and enzymes that display a wide range of pharmacological activities, mainly on nervous systems. Scorpion venoms have been characterized and many of their components bind specifically to ion channels, resulting in neurotoxic effects. Also enzymes that show gelatinolytic activity have been detected in *Tityus bahiensis* and *Tityus serrulatus* venom. These enzymes can be involved in tissue permeabilization and toxin processing. In this work we report the identification of hyaluronidase and fibrinogenolytic enzymes from the South American scorpion *Tityus serrulatus*. Initially, the venom was submitted to gel filtration on Sephadex G-50. The fractions active for hyaluronidase and fibrinogenolytic activities were submitted to ânion-exchange chromatography on HPLC system (column resource Q). SDS-PAGE zymogram tests using hyaluronic acid as substrate showed the presence of hyaluronidase in the crude venom and in several fractions. Similar results were obtained when fibrinogen was incubated with the venom at 37^{o}C. These results demonstrate the presence of hyaluronidase and fibrinogenolitic enzymes in the venom of *T. serrulatus*.

KEY WORDS: *Tituys serrulatus*, arthropod venoms, enzymes, hyaluronidase, fribrinogenolic activity

FINANCIAL SUPPORT: FAPEMIG, CNPq, FUNED

CORRESPONDENCE TO: MÁRCIA H. BORGES, Laboratório de Bioquímica e Química de Proteínas, Diretoria de Pesquisa e Desenvolvimento, Fundação Ezequiel Dias. Belo Horizonte, MG, Brasil. Email: marcia.borges@funed.mg.gov.br

NADPH-DIAPHORASE HISTOCHEMISTRY AND CYTOCHEMISTRY IN RAT BRAIN UNDER THE EFFECT OF CIRCULATING NEUROTOXINS

CRUZ-HÖFLING MA *(1), RAPÔSO C. (1), ZAGO G.M. (1,2), LE SUEUR, L.M. (3), FONTANA K. (1,2), ROCHA T. (1)

(1) Depto de Histologia e Embriologia, IB, (2) Depto de Farmacologia, FCM, UNICAMP, Campinas SP, 3Dept Ciências da Saúde, UNIFESP, Santos SP – Brasil

Neurotoxins may act on specific neural circuits inducing increase or decrease of neurotransmitters release, among which nitric oxide (NO). We recently demonstrate that *P nigriventer* spider venom (PNV) increases the permeability of blood brain barrier in hippocampus and cerebellum of rats, and activates neuronal pathways in motor- and acute stress related areas, all of which also expressed nNOS. Here, we investigated the NADPH-diaphorase (nicotinamide adenine dinucleotide phosphate) activity as a tool to add new insights on NO involvement in the central action of PNV. Adult rats (200-250 g) received systemic injection of PNV (0.85 mg/ml) or sterile saline and were sacrificed by perfusion after 3 h, 1, 5 and 9 days (n=8). Histochemistry (light microscopy-LM) and cytochemistry (transmission electron microscopy-MET) enzymatic reaction for NADPH-d were done. LM study was performed in 10 µm thick freeze sections, and MET reaction was done on 1.5 mm width tissues blocks, both incubated at 37ºC in a medium containing the reagents. Controls of the reaction were carried out omitting NADPH. The results showed that i.v. injection of PNV increased NADPH-d in cortex, hippocampus, thalamus and hippothalamus. This increase was more remarkable at 3 h and 1 d after envenoming. The cellular distribution of NADPH-d activity was found in neurons, astrocytes, microglia, pericytes and endothelial cells. Subcellular distribution included the cytosol and a discrete reaction attached to membranes of endoplasmic reticulum, mitochondria, Golgi complex and nuclear envelope. Control rats showed very weak presence of NADPH-d activity. We conclude that the spider venom neurotoxins, some of which sodium channels activating- or delaying inactivating-neurotoxins are involved in these effects. The mechanism apparently involves humoral NO signaling through an endothelium-dependent mechanism, since the NADPH-d activity peaked at a time the venom is still systemic.

KEY WORDS: Nitric oxide, venom, NADPH-d

CORRESPONDENCE: hofling@unicamp.br

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C-FOS AND N-NOS REACTIVE NEURONS IN RESPONSE TO CIRCULATING *Phoneutria nigriventer* SPIDER VENOM

RAPÔSO C. (1), ZAGO G.M. (1,2), MELO L.L. (3), CRUZ-HÖFLING M.A. (1)

(1) Dept Histology Embryology, IB, (2) Dept Pharmacology, FCM, UNICAMP, Campinas, SP, (3) Lab Neuropsychopharmacology, São Francisco Univ, Bragança Paulista, SP, Brazil

Drugs and neurotoxins activate specific neural circuits by increase or decrease neurotransmitters release (e.g. nitric oxide, NO), and by induction of immediate early genes (e.g. FOS). *P. nigriventer venom* (PNV) was shown to impair the microtubule-dependent transcellular barrier of blood-brain interface, but no visible alterations were seen in central neuronal cells. For clearing the central action of PNV we assess the distribution of highly activated neurons in the subcortical and cortical areas of the CNS 2h after intravenous injection of PNV, using FOS immunolabeling and the production of NO by immunodetection of neuronal nitric oxide synthase (nNOS). Male Wistar 7–10-week-old rats were divided into: a) rats received 0.5 ml 850µg/Kg PNV in the tail vein; b) rats were given 0.5ml 0.9 % saline solution; c) non-envenomed rats were kept intact until sacrifice (n=5/group). Acoustic, visual, and olfactory stimuli in the room were kept to a minimum, since FOS is a sensible marker. The PNV sublethal dose produced in the rats excitatory signs of salivation, lachrymation, tremors, flaccidity followed by spastic paralysis of the hindlimbs and convulsion. After anesthesy and perfusion fixation, the brain was removed and freezed. 30µm cryostat sections were serially collected at a 600µm distance for free-floating immunohistochemical reaction. FOS positive neurons predominate at motor-related areas: dorsolateral and ventral periaqueductal gray matter, frontal and parietal motor cortex, periventricular thalamic nucleus, and acute-stress-related ones: periventricular thalamic nucleus and lateral septal nuclei. nNOS positive neurons predominate at the periventricular thalamic nuclei, followed by dorso lateral periaqueductal gray matter and parietal cortex motor area. We conclude that those motor- and acute stress-related areas may represent a key-neuronal pathway in PNV envenoming mechanism. The coincident localization of nNOS and FOS in same anatomic regions suggests a modulatory role of NO in the toxicant stimulus.

KEYWORDS: brain, toxin, convulsion

CORRESPONDENCE TO: hofling@unicamp.br

FINANCIAL SUPPORT: FAPESP, CNPq, FAEPEX-UNICAMP

Loxosceles variegata: THE FIRST REPORT IN MINAS GERAIS STATE

CHATZAKI M. (1), PLAZZI M.I.A. (2), MELO M.M. (3), CHAVEZ-OLÓRTEGUI C.D. (1), KALAPOTHAKIS E. (1), DE MARIA M.(1)

(1) Instituto de Ciências Biológicas, Brazil, UFMG; (2) Centro de Zoonoses de Ituiutaba, MG, Brazil, (3) Escola de Veterinária, UFMG, Brazil.

The spider genus *Loxosceles* (ARANEAE, SICARIIDAE) is well represented in the area of Latin America. 36 out of the 101 species known worldwide are recorded from this area. Until recently ten species were known to occur in Brazil and three (*L. similis* Moenkhaus, *L. laeta* (Nikolet) and *L. anomala* (Mello-Leitão)) in the region of Minas Gerais. This study reports the presence of a fourth species in the area of Ituiutaba, Minas Gerais, Brazil, *L. variegata* Simon, 1897, which was previously known as endemic in Paraguay. This is the first time *L. variegata* is reported in Brazil. As it was collected in high numbers by locals, it is considered as a permanent inhabitant of the area and not an accidental venue by neighboring areas of Paraguay. The biology, ecology and ethology of this species are not well known, therefore its relation to humans are not yet understood. Because *Loxosceles* venom is generally known for being toxic to humans, further pharmacological and toxicological analysis of the venom of this species is needed to investigate the potential risk to humans, as well as further insights on its distribution in Brazil.

KEY WORDS: *Loxosceles variegata*.

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CORRESPONDENCE TO: Prof. Dr. Evanguedes Kalapothakis, (31) 34992713.

THE PRELIMINARY ANALYSIS OF AN EXPRESSED SEQUENCE TAGS (ESTS) DATABASE FROM THE VENOM GLANDS OF *Loxosceles laeta* SPIDER

FERNANDES-PEDROSA M. F. (1,3), JUNQUEIRA-DE-AZEVEDO I. L. M. (2), KOBASHI L. S. (2), GONÇALVES-DE-ANDRADE R. M. (1), HO P. L. (2), TAMBOURGI D. V. (1)

(1) Laboratório de Imunoquímica, Instituto Butantan, SP, Brazil; (2) Centro de Biotecnologia, Instituto Butantan, SP, Brazil; (3) Departamento de Farmácia, Universidade Federal do Rio Grande do Norte, RN, Brazil.

The bite of belonging to the genus *Loxosceles* can induce a variety of clinical symptoms, including dermonecrosis, thrombosis, vascular leakage, haemolysis, and persistent inflammation. The causative factor is a sphingomyelinase D (SMaseD) that cleaves sphingomyelin into choline and ceramide 1-phosphate and has intrinsic lysophospholipase D activity toward LPC. In order to generate a global panorama of the transcriptional activity of spider venom glands we have constructed a plasmid cDNA library from *Loxosceles laeta* venom glands mRNA to generate an Expressed Sequence Tags (ESTs) database. Sequences from 3008 independent clones were assembled in 1357 clusters (326 contigs and 1031 singlets), represented the transcripts profile of this tissue. The first 820 clusters were analysed and the repertoire of putative toxins corresponded to 116 sequences (14%) of all the transcripts. Sphingomyelinases represented the most abundant transcripts with 57 clusters (49%). We also found others transcripts corresponding metalloproteinases, serinoproteinases, C-lectins, cystein-rich and inhibitors of toxins. Among the 390 clusters matching cellular proteins, the major part represents molecules involved in gene and protein expression, reflecting the specialization of this tissue for toxin synthesis.

KEY WORDS: *Loxosceles laeta*, expressed sequence tags (ESTs), transcriptome.

FINANCIAL SUPPORT: FAPESP, CNPq.

CORRESPONDENCE TO: MATHEUS DE FREITAS FERNANDES PEDROSA, Laboratório de Imunoquímica, Instituto Butantan, SP, and DFAR/UFRN, RN Brazil. Phone: + 55 11 3726-7222, 2231, Fax: + 55 11 3726-7222, 1505, +55 84 3215-4341. E-mail: mpedrosa@butantan.gov.br.

ACTION OF GD+3 AND ZN+2 IONS ON THE DERMONECROTIC ACTIVITY OF THE SMASES D FROM *Loxosceles* SPIDER VENOMS

FERNANDES-PEDROSA M. F. (1, 2), ANDRADE S. A. (1), TAMBOURGI D. V. (1)

(1) Laboratório de Imunoquímica, Instituto Butantan, SP, Brazil; (2) Departamento de Farmácia, Universidade Federal do Rio Grande do Norte, RN, Brazil.

Bites by *Loxosceles* spiders can produce severe clinical symptoms, including dermonecrosis, thrombosis, vascular leakage, hemolysis, and persistent inflammation. The causative factor is a sphingomyelinase D (SMaseD) that cleaves sphingomyelin into choline and ceramide 1-phosphate and has intrinsic lysophospholipase D activity toward LPC. We have cloned and expressed the fully active recombinant sphingomyelinases from *L. laeta* (SMase I) and *Loxosceles intermedia* (SMases P1 and P2). The recombinant toxins were endowed with all biological properties ascribed for the whole venoms, including dermonecrotic and complement-dependent haemolytic activities and the ability of hydrolysing sphingomyelin. The aim of this study was to investigate the effect of Gd+3 and Zn+2 ions on the dermonecrotic inducing activity of the *Loxosceles* SMases D. Samples of the SMases I, P1 and P2 were incubated with increased concentrations of Gd+3 and Zn+2 ions (10mM, 20mM, 40mM) for 30 min at 37°C and then tested for dermonecrotic activity on rabbit model. Data obtained showed that both ions were able to neutralise the necrotic reaction induced by SMase I from *L. laeta* and SMases P1 and P2 from *L. intermedia*. These data suggest that Gd+3 and Zn+2 ions can compete with Mg+2 present in the active site of the SMases D blocking their toxic activity.

KEY WORDS: *Loxosceles* spiders venoms, sphingomyelinase D, dermonecrosis, Gd+3 and Zn+2 ions.

FINANCIAL SUPPORT: FAPESP, CNPq.

CORRESPONDENCE TO: DENISE VILARINHO TAMBOURGI, Laboratório de Imunoquímica, Instituto Butantan, SP, Brazil. Phone: + 55 11 3726-7222, 2231, Fax: + 55 11 3726-7222, 1505. E-mail: dvtambourgi@butantan.gov.br.

SURAMIN AND POLYETHYLENE GLYCOL ANTAGONIZE THE MYOTOXIC EFFECT OF *Agkistrodon contortrix laticinctus* CRUDE VENOM AND ITS MYOTOXIN

SERAFIM A.D. (1), TOMAZ M. A. (1), WILLIAMS, K. (3), MURAKAMI, M.T. (2), ARNI, R.K. (2), OWNBY, C.L. (4), MELO, P.A. (1)

(1) Departamento de Farmacologia Básica e Clínica ICB, CCS, Universidade Federal do Rio de Janeiro, 21941-590, Rio de, Janeiro, RJ, Brazil, (2) Departament of Physics IBILCE/UNESP, São José do Rio Preto, SP, Brazil

(4) Department of Physiological Sciences, Oklahoma State University, Stillwater, OK 74078, USA

We assessed the effects of suramin and polyethylene glycol (PEG400) against the myotoxicity of *Agkistrodon contortrix laticinctus* (ACL) crude venom and its myotoxin on mouse isolated extensor digitorum longus (EDL) and neuromuscular diaphragm preparation. Isolated EDL were exposed to ACL crude venom and myotoxin (25 µg/mL) alone or together with suramin (10 and 30 µM) and PEG (10 and 30 mM). The rate of creatine kinase (CK) release from the muscles was measured, indicating tissue damage. Basal CK release from EDL was 0,43±0,06 U.g-1.h-1, and after 60 minutes of exposure to the myotoxin and treatments it increased to 13,76±3,76 (ACL myotoxin), 5,90±1,19 and 2,10±0,86 (myotoxin plus suramin 10 and 30 µM, respectively), and 8,87±3,63 and 5,80±2,04 (myotoxin plus PEG 10 and 30 mM, respectively). ACL crude venom (60 min) also induced an increase in the rate of CK release up to 32,07±5,43 U.g-1.h-1, which was significantly inhibited by PEG 100 mM (6,47±3,14 U.g-1.h-1). Mouse neuromuscular diaphragm preparation was exposed to ACL crude venom and venom plus suramin. The preparation was directly (muscle) and indirectly (nerve) stimulated, and twitch amplitude was recorded. The snake venom (12,5 and 25 µg/mL) decreased twitch amplitude down to 25% of the control in approximately 30 to 40 min in both protocols. Suramin prevented in a concentration-dependent fashion the venom's effect (20, 25 and 50 mM caused a 5, 40 and 100% inhibition on the depressing effect of ACL crude venom, respectively). Data show that suramin and PEG are able to prevent the myotoxic activities of ACL crude venom and its myotoxin in mouse isolated muscle and suramin prevents the neuromuscular effects of ACL crude venom.

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KEY WORDS Cooperhead venom; Myotoxicity; Suramin and polyethylene glycol

CORRESPONDENCE:pamelo@farmaco.ufrj.br

FUNCTIONAL DIVERSITY OF TOXINS FROM SPIDERS OF THE GENUS *Phoneutria*

CAMPOS F.V. (1), LÚCIO A.D. (1), MATAVEL A. (1), LEÃO R.M. (1), KUSHMERICK C. (1), CRUZ J.S. (1), CORDEIRO M.N. (2), RICHARDSON M. (2), DINIZ C.R. (1,2) AND BEIRÃO P.S.L. (1)

(1) Department of Biochemistry and Immunology, ICB-UFMG, Belo Horizonte, MG; (2) Fundação Ezequiel Dias, Belo Horizonte, MG, Brazil

Spiders of the genus *Phoneutria* are prevalent over wide areas of Brazil, and their venoms contain a rich variety of peptides, most with activities on ion channels. After the pioneering work of Dr. C. R. Diniz, a number of investigators have been engaged in the determination of the structural and functional diversity of toxins contained in the venom of the spider *Phoneutria nigriventer*. Five fractions of polypetide toxins (mw. 3.5-8.5 kDa) have been described in this venom, including an insect-specific toxin. More recently, a small (4 kDa) family of neurotoxins has been identified in three species of the same genus: *Phoneutria nigriventer*, *Phoneutria reidyi* and *Phoneutria keyserlingi*. Preliminary work suggested that these toxins act on ion channels. We have focused our interest in the mechanism of action of the toxins, by determining the channels they target and their mechanisms of action. We have used the patch clamp technique, in the whole cell configuration, and appropriate cell types to directly measure currents carried through Na, Ca and K channels, and have used biophysical and pharmacological markers to identify the subtypes of channels that were affected. Most of the toxins found in the fraction PhTx2 have strong inhibitory effect on Na channel inactivation. This effect can account for the prevailing excitatory effect of the crude venom. In contrast, fraction PhTx3 is more diverse, containing toxins that inhibit Ca channels (w-PnTx3-3) and A-type K channels (PnTx3-1). Toxins that belong to the 4 kDa family inhibit L-type Ca channels, with different efficacies (PRTx27C3>PNTx27C4≈PNTx26An0C3, toxin PKTx32C4 having no effect). Since the overall inhibition of Ca channels is frequency-dependent, we propose that PhTx2 toxins potentiate the effect of PhTx3 fraction.

KEY WORDS: spider venom, neurotoxin, peptide, ion channel

FINANCIAL SUPPORT: CNPq and FAPEMIG

CORRESPONDENCE TO: PAULO BEIRÃO, Depto. Bioquímica ICB-UFMG. Phone: 55 31 3499-2663 / Fax: 55 31 3499-2614; email: pslb@ufmg.br

PKTX, A NEUROTOXIN OF THE SPIDER *Phoneutria keyserlingi* THAT INTERACTS WITH SODIUM CHANNELS FROM INSECTS

DA COSTA P.R. (1), GOUVÊA DOS SANTOS R. (2), BORGES M.H. (3), RICHARDSON, M.(3), CORDEIRO, M.N.(3), PIMENTA A.M.C.(1), DE LIMA M.E. (1)

(1) Lab. Venenos e Toxinas Animais, Depto. Bioquímica e Imunologia, ICB, UFMG, Belo Horizonte, MG, (2) Lab. Radiobiologia, Centro de Desenvolvimento da Tecnologia Nuclear/CNEN, Belo Horizonte, MG, (3) Centro de Pesquisa e Desenvolvimento, Fundação Ezequiel Dias, Belo Horizonte, MG

Spider venoms contain a multitude of toxins that target membrane receptors and ion channels. PKTx a novel neurotoxin isolated from the spider *Phoneutria keyserlingi*, was partially sequenced and its primary structure shows similarities with a-like insecticidal toxins isolated from the venoms of spiders of the genus *Phoneutria*. In preliminary studies, intra-cerebral injections of PKTx in mice (3µg/animal) caused immediate agitation, spastic paralysis, tail elevation and death after 10 minutes. In order to identify and characterize the effects of PKTx, we investigated its toxicity in mice and house flies and we verified its possible interaction with voltage activated sodium channels (Na+v) on mammal and insect synaptosomes. Intra-thoracical injections of PKTx in house flies (*Musca domestica*) showed elicited movements of legs and mouth parts, extension and contraction of the proboscis, paralysis resulting in loss of ability to fly, and death of 30% of animals (dose level of 500 ng/ animal). Binding studies with radioiodinated toxins were carried out to determine the sites of interaction of PKTx on cockroach (*Periplaneta americana*) nerve cord synaptosomes. PKTx (5.10-7M) partially competed (42%) with 125I-Bom IV, an a-like toxin from the scorpion *Buthus occitanus mardochei*, for the binding on the site 3 of Nav channel present on cockroach nerve cord synaptosomes. In conclusion, our results indicate that PKTx interacts with sodium channels of insect excitable cell membranes, probably as the a-like toxins do. Other studies are in development in order to better characterize the interaction of PKTx with insect and mammals nervous system.

KEY WORDS: neurotoxin, *Phoneutria keyserlingi*, a-like toxin

FINANCIAL SUPPORT: CNPq and FAPEMIG.

CORRESPONDENCE TO: Maria Elena de Lima, Laboratório de Venenos e Toxinas Animais, ICB- UFMG, 55(31)34992659. Email: delima@icb.ufmg.br

EVALUATION OF NEUTRALIZING EFFECT OF POLYCLONAL AND MONOCLONAL ANTIBODIES AGAINST *Tityus serrulatus* VENOM *in vitro* AND *in vivo*

ALVARENGA L(1), KALAPOTHAKIS E (2); C. GRANIER (3), DE LIMA ME (1) , OLORTEGUI CC(1), SANTOS RG(4);

(1) Depto de Bioquímica-Imunologia (2) Depto de Biologia Geral- UFMG, Brazil (3) CNRS UMR5160 Centre de Pharmacologie et Biotechnologie pour la Santé Faculté de Pharmacie, Montpellier, France (4) Centro de Desenvolvimento da Tecnologia Nuclear, CDTN/CNEN, Minas Gerais, Brazil

The aim of the present study was to obtain neutralizing monoclonal antibodies (mAbs) against *Tityus serrulatus* venom by the fusion of SP2/0 murine myeloma cells and spleen cells from BALB/c mice immunized with a toxic fraction (TstFG50) of the *Tityus* venom conjugated to BSA with glutaraldehyde. A panel of 9 anti-TstFG50 secreting hybridomas was established. The capacity of mAbs to neutralize the TstFG50 toxic fraction toxic was determined by *in vivo* neutralization assays and by inhibition of the binding of 125I-TsVII to its site on rat brain synaptosomes. In this work we used an *in vivo* neutralization assay based on the envenomation with a radiolabeled version of the main toxic component of the venom, 125I-TsVII, after pre-injection of monoclonal antibodies. 125I-TsVII specific binding on synaptosomes was strongly inhibited by several monoclonal antibodies. *In vivo* biodistribution in mice showed that the radiolabeled TsVII accumulated in lung and heart tissue, cleared rapidly from the blood, and was excreted significantly via the kidneys. Monoclonal antibodies MAbTs1, which react with peptide 26 of TsIV (KKSKDKKADSGYSYW), peptide 30 of TsVII (KKGSSGYSAWPASYS) and peptide 31 of TsNTxP (KKGSSGYSAWPASYS), was able to inhibit 35% of the specific interaction of 125I-TsVII with synaptosomes and neutralize the specific uptake, *in vivo*, in target organs responsible for the envenomation symptoms. In this work we produced monoclonal antibodies capable to inhibit the specific interaction of the toxic protein (TsVII) with sodium channels present on brain synaptosomes and neutralize its *in vivo* uptake by target organs involved in the scorpion envenomation symptoms.

KEY WORDS: *Tytius* venom, monoclonal antibodies, neutralization

FINANCIAL SUPPORT: CNPq, CDTN/CNEN

CORRESPONDENCE TO: Raquel Gouvêa Dos Santos, Lab. Radiobiologia; CDTN/CNEN; C. P. 941; 30123-970; Belo Horizonte - MG – Brasil. santosr@cdtn.br

EFFECTS OF THE TITYUSTOXIN ISOLATED FROM *Tityus serrulatus* SCORPION VENOM IN PERFUSED RAT KIDNEY

ALVES R.S. (1), MARTINS R.D. (1), SOUSA D.F. (1), ALVES C.D. (1), APRÍGIO C.C. (1), QUEIROZ M.G.R. (3), MARTINS A.M.C. (3), TOYAMA M.H. (4), FONTELES, M.C. (1), MONTEIRO, H.S.A. (1)

(1)Department of Physiology and Pharmacology, (2)Department of Pathology and Legal Medicine and (3)Department of Clinical and Toxicology Analysis/ Federal University of Ceará, (4)Department of Chemistry, Sao Paulo State University.

Tityus serrulatus is the most dangerous scorpion species accounting for fatal stings, especially among children, in Brazil. The tityustoxin is a rich source of various polypeptides with diverse physiological and pharmacological activities that generally exert their action via target specific modulation of ion channel function. The aim of present work was to determine the effects of tityustoxin isolated from *Tityus serrulatus* (TsTx) venom in isolated perfused kidney from Wistar rats, as it was described by Fonteles *et al*., 1983 (1). The effects of 3µg/mL toxin concentration (n=6) were studied on perfusion pressure (PP), renal vascular resistance (RVR), urinary flow (UF), glomerular filtration rate (GFR), sodium (%TNa+), potassium (%TK+) and chloride (%TCl-) tubular transport. All experiments were preceded by 30 minutes of internal control (C). The data were analyzed by ANOVA and Student t-test with level of significance was set at *p<0.05. The TsTx increased the PP (C = 109.7 ± 0.75 vs. TsTx = 112.15 ± 0.17 mmHg*) and RVR (C = 5.95 ± 0.04 vs. TsTx = 6.13 ± 0.01mmHg.min-1.g-1.min*) at 60 min. The toxin also increased UF with maxim effect at 120 min (C = 0.10 ± 0.01 vs. TsTx = 0.13 ± 0.004 mL.g-1.min-1*). The toxin reduced %TNa+ (C = 71.92 ± 1.45 vs. TsTx = 62.3 ± 2.43%*) and %TCl- (C = 69.13 ± 1.68 vs. TsTx = 59.22 ± 2.35%*) at 90 min. In conclusion, TsTx altered the renal functional parameters evaluated and it is mainly responsible for the increase of urinary flow induced by crude venom.

KEY WORDS: scorpion, *Tityus serrulatus*, tityustoxin, renal effects.

REFERENCE: (1) FONTELES, M. C. et al. (1983). Am J Physiology 44: 191-197.

FINANCIAL SUPPORT: CNPq.

CORRESPONDENCE TO: MONTEIRO, Helena S. A. Department of Physiology and Pharmacology/ Federal University of Ceará. Phone: +55 85 33668247. Fax: +55 85 33668333. Email: renatasalves@gmail.com

ANALYSIS OF LUNG COMPLIANCE AFTER *Tityus serrulatus* ENVENOMATION IN ANESTHETIZED MICE UNDER MECHANICAL VENTILATION

PERES A. C. P. (1), NONAKA P. N. (1), FERRARI E. F. (1), CARREIRO DA COSTA R. S. (1), SILVA C. A. M. (2), AMORIM C. F. (1), TOYAMA M. H. (3), RIBEIRO W. R. (1), COGO J. C. (1), OLIVEIRA L. V. F. (1)

(1) Vale do Paraíba University - UNIVAP, IP&D, São José dos Campos-SP, Brazil; (2) Department of Respiratory Physiology, Brasília University - UNB, Brasília - DF, Brazil; (3) State University of São Paulo-UNESP, São Vicente-SP.

The severity of scorpion envenomation is related to the cardiorespiratory alterations it may provoke, with manifestations such as pulmonary edema and circulatory failure triggering death. This study aimed to test if this venom could induce lung compliance alterations after 3 h venom injection. Eighteen Swiss mice, were analysed twelve hours after intraperitoneal injection of saline (control group) or *T. serrulatus* crude venom (0,6 mg.g-1) The sub-lethal dose used in venom group, was determined by the LD50 previously found. The mechanical parameters were obtained by End Inspiratory Oclusion Method. The statistical analysis was carried through the Kolmogorov-Smirnov test for normality and the independent t-test in a significance level of 5%. Static elastance, dynamic elastance, ΔP1 and ΔP2 increased ($p<0,05$), after 3 hours venom injection. These finds are in part due to the fact that scorpion toxin induces pulmonary edema in humans and experimental animals, fact that could explain the increased viscous properties. Variations in the elastic and viscoelastic properties could be correlated with the severity of the pulmonary inflammation. According to the literature, histological examination of the lungs showed a slight or moderate edema characterized by intra-alveolar plasma leakage, alveolar wall thickening, areas of collapse and mononuclear inflammatory infiltration which can account for the reduction in lung elasticity and, therefore, lung compliance.

KEY WORDS: scorpion, venom, *Tityus serrulatus*, lung compliance, mechanical ventilation.

FINANCIAL SUPPORT: FAPESP, CAPES, UNIVAP.

CORRESPONDENCE TO: LUIS VICENTE FRANCO DE OLIVEIRA, IP&D – UNIVAP, São José dos Campos-SP, Brazil. Phone/Fax: + 55 12 39471144. Email: oliveira@univap.com

UNRAVELING TOXINS INVOLVED IN ENVENOMING BY *Lonomia obliqua* CATERPILLARS

RICCI-SILVA ME. (1), VALENTE RH. (2), LEÓN IR. (2), TAMBOURGI D V.(3) PERALES J (2), SIMONS MS. (1), CHUDZINSKI-TAVASSI AM (1).

(1) Laboratório de Bioquímica e Biofísica. Instituto Butantan, São Paulo, SP, Brasil. (2) Departamento de Fisiologia e Farmacodinâmica. Instituto Oswaldo Cruz - IOC-FIOCRUZ, Rio de Janeiro, RJ, Brasil. (3) Laboratório de Imunoquímica. Instituto Butantan, São Paulo, SP, Brasil

Some coagulation disorders have been reported after contact with *Lonomia obliqua* caterpillars. However, patients have the physiological coagulation parameters recovered after treatment with specific anti-serum produced in horses against *Lonomia obliqua* bristle extract. Previous studies characterized procoagulant proteins (Lopap and Losac) from its bristles extract. The aim of this study was to identify the immunogenic components specially related to haemostasis by proteomic and immunological approaches. By these analyses, it was observed that the most expressed proteins in *Lonomia obliqua* extract were lipocalins, therefore they may have an important role in the envenomation process. Other identified components were serinoproteinase and proteins related to the cuticle, as it could be expected, once bristles extract was used for production of the antiserum.

KEY WORDS: *Lonomia obliqua*, haemostasis, proteomic, lipocalins

FINANCIAL SUPPORT: FINANCIAL SUPPORT: FINEP-COINFAR - CNPQ

CORRESPONDENCE TO: Maria Esther Ricci-Silva. Lab. de Bioquímica e Biofísica, Instituto Butantan, SP, Brasil. Phone 55 (11) 3726-7222 r2109.E-mail: esthericci@butantan.gov.br

PROTEOMICS ANALYSIS OF THE SALIVARY SECRETIONS FROM THE *Amblyomma cajennense* TICK (ACARI: IXODIDAE) (FABRICIUS: 1787)

SIMONS S. M. (1), RICCI-SILVA M. E. (1), VALENTE R. H. (2), LEON I. R. (2), PERALES J. (2), CHUDZINSKI-TAVASSI A. M. (1).

(1) Laboratório de Bioquímica e Biofísica. Instituto Butantan, São Paulo, SP, Brasil. (2) Departamento de Fisiologia e Farmacodinâmica. Instituto Oswaldo Cruz - IOC-FIOCRUZ, Rio de Janeiro, RJ , Brasil.

Ticks, such as *Amblyomma cajennense*, are the main vectors for several pathologies, such as babesiosis, Maculosa Fever and Lyme-Simile disease. Bloodsucker' saliva contains a complex mixture of bioactives components, which interfere in their host's physiological mechanisms. In this work, we used proteomics approaches to investigate the protein profile in the tick's saliva. The saliva was collected according to the method described by Kaufman, then the sample was precipitated by TCA to perform the bidimensional electrophoresis (2D) analysis. The proteins from 2D gel were revealed by silver staining (using a protocol compatible with mass spectrometry analysis), the selected spots were removed, digested by trypsin and submitted to mass spectrometry analysis (MALDI-TOF-TOF). The protein identification was performed by carrying out PMF and/or MS/MS analyses, and the sequences were searched in MASCOT data bank. The 2D gel of reached by the crude saliva showed 110 spots, the pI range was between 3.5 and 9.5; the range of molecular mass was from 12 to 160 kDa. Previously our group identified some anticoagulant proteins from the tick saliva, and among them low (about 7,5-15 kDa) and high (about 60-70 kDa) molecular weight proteins were found, due to this we select the same range of molecular mass to sequence the proteins and the preliminary spots selected were about 14 KDa, the protein analysis by MALDI-TOF mass spectrometry identified a hemoglobin-like protein (alpha and beta chains), the hypotheses of this protein belong to the host origin is not discarded; however the other analysis will be done to check out the obtained data and to continue the sequence of the other spots.

KEY WORDS: *Amblyomma cajennense*, ticks, crude saliva, proteomic, anticoagulant

FINANCIAL SUPPORT: FAPESP, FINEP-COINFAR

CORRESPONDENCE TO: Simone Michaela Simons, Lab. de Bioquímica e Biofísica, Instituto Butantan, SP, Brasil. Phone 55 (11) 3726-7222 r 2109. E-mail: Smsimons@butantan.gov.br

DO PERIPHERAL NK1 RECEPTOR AND SUBSTANCE P PLAY AN IMPORTANT ROLE IN *Phoneutria nigriventer* VENOM-INDUCED ITCH IN THE MICE DORSAL SKIN?

COSTA S.K. (1), STARR A. (2), HYSLOP S. (3), GILMORE D. (2), BRAIN S.D. (2)

(1) Department of Pharmacology (ICB-I), University of São Paulo, São Paulo, Brazil; (2) Cardiovascular Division, King's College London, UK; (3) Department of Pharmacology (FCM, UNICAMP), Campinas-SP

Pain and itch sensations are induced by depolarization of C-fibre nerves and possibly other types of fibres. We have evidence from several species, including mice, that skin plasma extravasation induced by the *Phoneutria nigriventer* spider venom (PNV) is dependent on tachykinin NK1 receptors. We have now investigated the itching measured as bouts of scratching in response to intradermal (i.d.) PNV in wildtype (WT) and NK1 receptor knockout (KO) mice. Mice were given a single i.d. injection (0.05 ml) of test agent or vehicle into the shaved dorsal skin, in the intercostal region, in a randomized way. The bouts of scratching were recorded in a blinded manner for 60 min. Oedema formation was concomitantly assessed by the extravascular accumulation of 125I-albumin. The i.d. injection of either substance P (at a high dose of 100 nmol/site) or PNV (0.3-10 mg/site) induced oedema formation in WT but substantially less was observed in NK1 KO mice, as previously reported. PNV also induced scratching, but significantly less scratching was observed in NK1 KO with WT mice. In contrast, SP did not induce significant scratching at amounts up to 100 nmol in WT mice. Experiments with an NK1 receptor antagonist SR140333 (at doses that blocked PNV-induced oedema) revealed that whilst a local co-injection (1 nmol) in WT mice had no effect on PNV (3 mg/site)-induced scratching (18.5 ± 3.7 vs. 14.4 ± 3.5 bouts, mean ± S.E.M., n=5-7), systemic treatment with SR140333 (120 nmol/kg, i.v.) significantly inhibited scratching (14 ± 3.5 vs. 3.1 ±1.2* bouts, n=4-6). These results indicate that NK1 receptors are involved in mediating PNV-induced scratching and that the location of the receptors is unlikely to be skin. Thus, a distinct separation between endogenous microvascular and PNV nociceptive NK1-dependent effects is suggested.

KEY WORDS: *Phoneutria nigriventer*, spider, itch, NK1 receptor, oedema

FINANCIAL SUPPORT: BHF (U.K.), CNPq, FAPESP

CORRESPONDENCE TO: S Costa. Dept of Pharmacology, ICB-USP, Phone: (+55) 11-30917320. E-mail: scosta@icb.usp.br

LOCAL INFLAMMATORY EFFECTS EVOKED BY *Polistes lanio lanio* WASP VENOM IN THE MICE DORSAL SKIN: ROLE OF TACHYKININS

YSHII LM (1), HYSLOP S (2), BRAIN SD (3), TAM C (3), RIBELA MTP (4), MUSCARÁ MN (1); COSTA SKP (1)

(1) Dept. of Pharmacology, ICB-USP, São Paulo; (2) Dept. of Pharmacology, Unicamp, Campinas; (3) Cardiovascular Division, NHH, King's College London; (4) Ipen, São Paulo

Polistes lanio lanio wasp venom (PLLv) causes neurogenic oedema via a tachykinin NK1 receptor-mediated mechanism in mouse dorsal skin (1). Using venom obtained by a different extraction method, this effect was not inhibited by an NK1 receptor antagonist SR140333. We used normal C57BL/6 as well as NK1 and TRPV1 receptor knockout (KO) mice to compare the oedematogenic effects of venom prepared by these two methods. Venom (PLLv1) was obtained as described (1). In a new method, the venom sacs were removed along with the sting. The sting was inserted into a polyethylene cannula and the venom (PLLv2) was expelled into the cannula by lightly compressing the venom sac. Normal or NK1 and TRPV1 receptor KO mice (25-30 g) were anaesthetized with urethane. The venom or vehicle was injected i.d. into the shaved dorsal skin. After 30 min, the injected site was removed. Oedema formation was assessed by the extravascular accumulation of 125I-albumin in the skin compared to plasma. The results were expressed as the mean ± SEM and were compared by ANOVA plus Bonferroni's test. Results show PLLv2 (0.3 – 10 mg/site) caused potent, dose-dependent oedema in mouse skin. In contrast to PLLv1, the PLLv2-induced effect was not affected by SR140333, but was markedly reduced by the histamine H1 receptor antagonist, pyrilamine. The PLLv2-induced oedema in normal mice was similar to that in NK1 and TRPV1 KO mice. We conclude that PLLv2 caused oedema more potent to that of PLLv1. In addition, the PLLv2 obtained by the new method was devoid of the neurogenic effect, suggesting that the previous method resulted in contamination of the venom by tachykinin-like components from glandular tissue.

KEY WORDS: Polistes, wasp, oedema, mice, TRPV1, NK1 receptor

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CORRESPONDENCE TO: S Costa. Dept of Pharmacology, ICB-USP. Phone/Fax: (+55) 11-30917320. E-mail: scosta@icb.usp.brEmail: prianti@univap.br.

PHOSPHOLIPASE A2 AND MASTOPARAN FROM *Polybia paulista* WASP VENOM ON MICE SKELETAL MUSCLE

ROCHA T. (1), BARROS L.L.S. (1), SANTOS L.D. (2), DE SOUZA, B.M. (2), PALMA M.S. (2), CRUZ-HÖFLING M.A. (1)

(1) Dept. Histology & Embryology, Inst. Biology, UNICAMP, Campinas, SP, Brazil, (2)Center of Studies of Social Insects, Inst. Biosciences, UNESP, Rio Claro, SP, Brazil.

Allergic reactions caused by wasp bites in humans is well-known. However, the action of the venom on the muscle fibers still remains unclear. Here we compared the myotoxic potency of mastoparan and phospholipase A2 (PLA2) (2.5 ug/uL) from *P. paulista* venom injected (i.m.) in the tibial anterior (TA) muscle of Balb/c mice. Time course of the changes were followed at degenerative (3 and 24 h) and regenerative (3 and 7 d) periods (n=6). Saline-injected groups were used as controls. The muscles were fixed in 4% paraformaldehyde and processed for embedding in paraffin. Histological sections (5 um) were stained with Hematoxylin-Eosin and the percentage of the damaged fibers was calculated and statistically analysed ($p<0.05$). A median of 900 fibers were counted. The controls showed normal morphology. At 3 h, 1.6% of the muscle fibers injected with PLA2 presented densely-clumped myofibrils intermingled with empty-looking sarcoplasmic areas. Inflammatory infiltrate foci were widespread and strong mainly during the intermediate phase (24 h), when just 1.1% of the muscle fibers were affected. In contrast, 27.3% (3 h) and 17.7% (24 h) of the muscles fibers were affected by mastoparan. In the late phase (3 d), whereas the muscles treated with PLA2 do not present small regenerating cells, but 1% of affected muscles fibers, the muscles treated with mastoparan presented 43.4% of regenerating cells against 6.7% of damaged cells. In the final phase (7 d), whilst regenerating area corresponded to 5.4% for PLA2, it represented 66.5% for mastoparan. The inflammatory infiltrate in the muscles treated with PLA2 still persisted. We conclude that despite PLA2 is poorly myotoxic, its inflammatory effect predominates over mastoparan. Interestingly, the regenerating process is faster in the mastoparan, than in PLA2's.

KEY WORDS: caspases, mitochondrial damage, immunohistochemistry, western blotting

SUPPORT: CNPq, FAPESP, CAPES, Instituto Milênio

CORRESPONDENCE: THALITA ROCHA, DHE, IB, UNICAMP, CP 6109, 13083-863 Campinas, SP, Brazil, 55 19 35216250, tharocha@yahoo.com

MASTOPARAN FROM *Polybia paulista* WASP VENOM IN SKELETAL MUSCLE APOPTOSIS

ROCHA T. (1), BARROS L.L.S. (1), DE SOUZA, B.M. (2), PALMA M.S. (2), CRUZ-HÖFLING M.A. (1)

(1) Dept. Histology & Embryology, Inst. Biology, UNICAMP, Campinas, SP, Brazil; (2) Center of Studies of Social Insects, Inst. Biosciences, UNESP, Rio Claro, SP, Brazil.

Our previous studies with light and transmission electron microscopy (TEM) showed that the mastoparan (I-N-W-L-K-L-G-K-M-V-I-D-A-L-NH2) from *P. paulista* wasp venom causes marked mitochondria changes together with myonecrosis in the tibial anterior (TA) muscle of Balb/c mice. However, although the pathologic states of the necrotic process of the fibers caused by mastoparan closely resemble those by snake venoms, apoptotic-looking fibers were seen after i.m. injection of the *P. paulista* wasp whole venom. In this study, we investigate possible apoptotic events in different time intervals (from 3 h to 21 d) after TA i.m. injection of 2.5 ug/uL mastoparan by examining the expression of caspases 9 and 3 seen by Western Blotting (WB) and Immunohistochemistry (IH). Saline solution-injected muscles were used as control. Controls were negative for caspase 9 and 3 in all periods. Myonecrotic fibers of mastoparan injected TA was strongly positive for caspase 9 and 3 which appeared expressed in the empty-looking sarcoplasmic areas at 3 and 24 h, but was negative at 3, 7 and 21 days. WB of the proteins confirmed the IH results, since a significant reduction of the bands were seen 3, 7 and 21 d in relation to that of 3 and 24 h. We concluded that mastoparan can mediate cell death by apoptosis involving a caspase 9 and 3 activating mechanism at early (3 h) and intermediate (24 h) phases, probably explaining the mitochondrial alterations seen by TEM. Mastoparan has been reported to facilitate the opening of the mitochondrial permeability transition pore through an apparent bimodal mechanism of action. The decrease of caspase 9 expression, seen on late (3 d) and final phases (7 and 21 d) probably reduced the caspase 3 activation mechanisms and consequently in the death by apoptosis. These phases correspond to regenerative periods of muscle fibers after toxin effect.

KEY WORDS: caspases, mitochondrial damage, immunohistochemistry, western blotting

SUPPORT: CNPq, FAPESP, CAPES, Instituto do Milênio

CORRESPONDENCE: THALITA ROCHA, DHE, IB, UNICAMP, CP 6109, 13083-863 Campinas, SP, Brazil, 55 19 35216250, tharocha@yahoo.com

PROTEOLYTIC ACTIVITY OF AMAZONIAN SCORPION *Tityus metuendus* VENOM (SCORPIONES: BUTHIDAE)

ARAÚJO T. F. (1, 3), HIGA A. M. (1, 3), NORONHA M. D. N. (1, 3), MARTINS M. J. P. (2), MUNIZ E. G. (3), MEDEIROS B. M. (4), AGUIAR N. O. (4), LOPEZ-LOZANO J. L. (1,3).

(1) Programa de Pós-Graduação em Biotecnologia e Recursos Naturais – Universidade do Estado do Amazonas; (2) Programa de Pós-Graduação em Biotecnologia – Universidade Federal do Amazonas; (3) Fundação de Medicina Tropical do Amazonas; (4) Curso de Ciências Biológicas da Universidade Federal do Amazonas.

Neurotoxin peptides are the main constituents of scorpion venoms. Few studies about Amazonian scorpion neurotoxins have been reported. Enzymes studies of scorpion's venoms are necessaries to better understand of biological function this venom. *Tityus metuendus* specimens are principal responsible of scorpionism in Manaus region. The aim of this study was research in *Tityus metuendus* venom toxins with phospholipasic and proteolytic activities. The scorpions were captured in Manaus region (Amazonas State – Brazil) and the venom was obtained by low voltage electric stimulation of telson venom gland. Pool sample venom (90 µg) was submitted to SDS – PAGE reduction or non reduction conditions to detected ≥ 14kDa proteins constituents. The phospholipasic activity was tested using 3% egg yolk incorporated in 1% agarose plate gel method in buffer PBS 0,10 mM pH 8,1 and 0,09 mM CaCl2. Proteolytic zimogram activity profile was obtained using different substrates (casein, hemoglobin, gelatin, and fibrinogen) in buffer solution pH 6. 5 – 8. 5 ranges. Gelatin and casein substrates were degraded by proteolytic activity of venom using glicine 100 mM pH 8. 3 buffer. The zimogram profile suggests gelatinolytic and caseinolytic enzymes with 23 kDa and 50 kDa respectively. These enzymes were inhibited by serine protease inhibitors. A Phospholipasic activity was not detected. Purification of proteolytic enzymes are in curse to know structure / biological function relationship.

KEY WORD: scorpion, *Tityus metuendus*, serine proteases, Amazonia.

FINANCIAL SUPPORT: CNPQ, FAPEAM, FMTAM, FINEP.

CORREPONDENSE TO:THIAGO FERREIRA DE ARAÚJO, Centro de Ofidismo Prof. Paulo Friedrich Bürheim, Fundação de Medicina Tropical do Amazonas, Manaus, Brasil. Phone: + 55 92 3238 42 94. E-mail: thiago_biologo@hotmail.com.

A NOVEL DRUG LEAD TO ANTIHYPERTENSIVE AGENT FROM *Tityus serrulatus* VENOM

VERANO-BRAGA T. (1,2), SILVA D.M.R. (4), SANTOS C.F.F. (4), BEMQUERER M.P. (2,3), SANTOS R.A.S. (4), BOUGIS P.E. (5), MARTIN-EAUCLAIRE M.F. (5), DE LIMA M.E. (1,2) , PIMENTA A.M.C. (1,2)

(1) Núcleo de Biomoléculas, (2) LVTA and (3) Lab. de Enzimologia e Físico-Química de Proteínas, Depto. de Bioquímica e Imunologia e (4) Lab. de Hipertensão, Depto. de Fisiologia e Biofísica, ICB, UFMG, Belo Horizonte, Brazil.

(5) CNRS-FRE 2738, Ingénierie des Protéines, Université de la Méditerranée, UMR6560, IFR Jean Roche, 13916 Marseille, France

Facilities in using micro-scale analytical techniques have led to a novel approach to prospect bioactive molecules in animal venoms. By this approach, we were able to find a new structural family of peptides in the venom of *Tityus serrulatus*, named TsHptP (*T. serrulatus* Hypotensive Peptides). Structurally, these are random-coiled linear peptides, ranging from 2.5 to 3 KDa and have a similar bradykinin-potentiating peptide (BPP) amino acid signature. TsHpt-I, a member of this peptide family, was able to potentiate the hypotensive effects of bradykinin (BK) in normotensive rats. To optimize the pharmacokinetics and the stability of TsHpt-I, few synthetic analogs were constructed using the TsHpt-I as a template. These analogs held the BK-potentiating effect. A relevant hypotensive action, which is independent on BK, was observed in all of these analogs, indicating that they are themselves hypotensive agents. We used hypertensive rats strains to study this hypotensive effect and it has been shown that these analogs induces a strong and long-lasting hypotensive effect. To evaluate this action, we examined the vasorelaxation effect of aortic rings derived from male Wistar rats. The analogs were able to induce ± 20% of vasorelaxation (10-7 M). One of these analogs was orally administrated in SHR rats and was able to reduce the blood pressure, indicating that it is stable and can be absorbed in the gastrointestinal tract. Beside these peptides have a similar amino acid sequence with the classical BPPs, some differences in the primary structure (data not shown) may be crucial to the new pharmacological effects observed in this peptides.

KEY WORDS: Bradykinin Potentiating Peptide, *Tityus serrulatus*, hypotensive peptides

FINANCIAL SUPPORT: CNPq; FAPEMIG; FINEP

CORRESPONDENCE TO: Adriano Pimenta, E-mail: apimenta@icb.ufmg.br

STRUCTURAL AND ULTRASTRUCTURAL CHARACTERIZATION OF THE VENOM GLAND OF *Vitalius dubius* (ARANEAE, THERAPHOSIDAE)

ROCHA E SILVA T.A.A. (1), CRUZ-HÖFLING M.A. (2), COLLARES-BUZATO C.B. (2), HYSLOP S. (1)

(1) Dept. of Pharmacolgy, Faculty of Medical Sciences and (2) Dept. of Histology and Embryology, Institute of Biology, State University of Campinas (UNICAMP), Campinas, SP, Brazil.

Tarantulas (family Theraphosidae) produce venom to subdue prey and for defense. We have previously described the general organization and histology of the venom apparatus of *Vitalius dubius*, a tarantula species found in southeastern Brazil [1]. In this work, we provide additional findings on the organization and ultrastructure of this venom gland. Histologically, the venom gland consisted of an external layer of striated muscle lined internally and externally by an elastin-rich basal membrane that supported the secretory epithelium. The venom-producing epithelium formed a complex network of anastomosing nuclei adjacent to the basal layer and long cytoplasmic elongations that extended into the lumen to form large venom-containing vesicles. Fluorescence confocal microscopy revealed abundant filamentous actin that contributed to the epithelial organization and shape. Transmission electron microscopy showed abundant smooth and rough endoplasmic reticulum, Golgi complexes and mitochondria. Ultrastructurally, glands 1, 3, 7 and 15 days after venom milking showed epithelial changes that included a looser nuclear organization, alterations in the abundance of endoplasmic reticulum and Golgi apparatus, and the formation of venom-containing vesicles.

KEY WORDS: tarantula, regeneration, ultrastructure, venom gland.

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CORRESPONDENCE TO: THOMAZ A. A. ROCHA E SILVA. Department of Pharmacology, Faculty of Medical Sciences, State University of Campinas (UNICAMP), PO Box 6111, 13083-970, Campinas, SP, Brazil. Tel.: +55-19-3521-9535, Fax: +55-19-3788-9536, E-mail: taars@unicamp.br; hyslop@fcm.unicamp.br

BIOCHEMICAL AND PHARMACOLOGICAL CHARACTERIZATION OF *Vitalius dubius* (ARANEAE, THERAPHOSIDAE) VENOM

ROCHA E SILVA T.A.A. (1), FRANCHI JR. G.C.(2), NOWILL A. E. (2), HYSLOP S. (1)

(1) Dept. of Pharmacology and (2) Onco-Hematological Child Research Center, Faculty of Medical Sciences, State University of Campinas (UNICAMP), Campinas, SP, Brazil.

Tarantula venoms are a rich source of proteins and peptides with novel pharmacological activities. In this work, we examined the composition and some biological activities of venom from the Brazilian tarantula *Vitalius dubius*. Venom was obtained by electrostimulation and contained high hyaluronidase activity (275±24 turbidity reducing units/mg of protein; mean±SEM, n=4) but no significant proteolytic activity towards casein, collagen or elastin. SDS-PAGE (10% and 20% gels) revealed proteins of 14 to 160kDa and two bands of peptides (2-4kDa and 6-12kDa). Fractionation of the venom by reversed phase chromatography resulted in three major and four minor peaks. The venom reacted in ELISA with affinity purified IgG from arachnidic antivenom (Butantan Institute) raised against spider (*Phoneutria nigriventer* and *Loxosceles gaucho*) and scorpion (*Tityus serrulatus*) venoms. Immunoblots detected proteins of 40 to 100 kDa. Venom dose-dependently increased the vascular permeability in rat skin (53±3, 100±6, 153±8, 165±9 and 202±10 ml of plasma for 1, 3, 10, 30, and 100 mg of venom/site, respectively; n=6) but did not contract isolated guinea-pig ileum (up to 100 mg/ml) or significantly alter the dose-response curves to acetylcholine or serotonin; however, it potentiated the maximum response to bradykinin from 25 to 200% over control. The venom (up to 200 mg/ml) was not hemolytic to rat erythrocytes but was cytotoxic to the leukemic cell line K562 (maximum mortality of 64±3% after 72h with 300 mg of venom/ml, n=3). *Vitalius dubius* venom causes edema and cytotoxicity and contains various proteins and peptides, some of which share immunological similarity with other arachnid venoms.

KEY WORDS: hyaluronidase, tarantula, vascular permeability, venom.

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CORRESPONDENCE TO: THOMAZ A.A. ROCHA E SILVA, Dept. of Pharmacology, Faculty of Medical Sciences, State University of Campinas (UNICAMP), PO Box 6111, 13083-970, Campinas, SP, Brazil. Fax: +(55) (19) 3289-2968, E-mail: taars@unicamp.br; hyslop@fcm.unicamp.br

CLINICAL ASPECTS OF UNCOMMON ENVENOMING PROVOKED BY ARTHROPODS: TOXINS FOR KNOWING

HADDAD JR, V(1,2).

(1) - Faculdade de Medicina de Botucatu, Universidade Estadual Paulista. (2) - Vital Brazil Hospital, Butantan Institute, São Paulo State, Brazil.

The arthropods constitute the most numerous group of animals of the Nature. Several of them present toxins as mechanisms of attack and defense, being able to cause injuries in human beings. There is clinical interest and an immense pharmacological potential in these toxins, practically unexplored. They cause interesting and curious skin lesions, which are important for the dermatologist. METHODS: The accidents had been selected in the casuistry of the author, in accordance with the rarity of the occurrence and the potential interest for toxinologists. RESULTS/DISCUSSION: the clinical aspects of envenoming for Millepede or Diplopoda are presented. These animals can eject irritating fluids that cause brown pigmentation and occasionally severe inflammation and blisters in the skin. The stink bugs (Pentatomidae) have glands that produce a mixture of hidrocarbonates that function as a repellent of predators and paralysis in prey. In humans, the secretion causes inflammatory skin lesions. The effects of the secretion are described in the first observation of lesions caused by Pentatomidae in humans. Finally, an accident caused for a tocandira ant is presented (Paraponera clavata). The tocandira is a giant ant which sting provokes violent pain and systemic manifestations in the victim. The intention of this presentation is to demonstrate uncommon injuries caused by venomous and poisonous arthropods and to offer new paths for interested on the involved toxins, poorly studied until today.

KEY WORDS: venomous animals, arthopods, toxins, stink bugs.

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CORRESPONDENCE TO: Vidal Haddad Junior - 18618-000 – Botucatu – SP – Brasil (haddadjr@fmb.unesp.br)